ACTH

Proceedings of the Sheffield Corticotropin Conference

HELD AT
CREWE HALL, UNIVERSITY OF SHEFFIELD
7th & 8th JANUARY, 1960

EDITOR: DR. H. F. WEST



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INTRODUCTION

A group of United Kingdom physicians and laboratory research workers particularly interested in ACTH met in Sheffield on 7th & 8th January 1960. My presence at the conference arose through personal contact with the convener, Dr. H. F. West. The proceedings of the conference were recorded for private use only but as information about ACTH and its clinical use was so meagre and so scattered in the literature I sought and obtained the permission of the participants to arrange for the publication of the proceedings.

It is anticipated that all physicians and laboratory research workers who have an interest in ACTH will find the proceedings as a whole, of interest, and will find some aspects of particular importance to them.

F. Paulsen.

PARTICIPANTS IN THE CONFERENCE

Dr. E F. B Cadman	Southport Infirmary.
Dr. J. Cates	The Royal Infirmary, Bristol.
Dr. T. M. Chalmers	The Middlesex Hospital, London.
Dr. C. L. Cope	Postgraduate Medical School, London.
Dr. J. L. Crammer	The Royal Free Hospital, London
Dr Beryl Davies	Imperial Cancer Fund Laboratories, London.
Dr. P. S. Davis	West London Hospital Medical School
Dr. H. B. F. Dixon	Dept. of Biochemistry, Cambridge
Mr A. P. M. Forrest	Western Infirmary, Glasgow.
Dr. K. Fotherby	Medical Research Council, Clinical Endocrinology Research Unit, Edinburgh.
Dr. J. K Grant	University Dept. of Biochemistry, Edinburgh.
Dr. R. Hall	University Dept of Medicine, Newcastle.
Dr. J. T Hemingway	Addenbrooks Hospital, Cambridge
Dr. G Holti	Royal Victoria Infirmary, Newcastle
Dr. L E Houghton	Harefield Hospital, Middlesex.
Dr. V. H T. James	St Mary's Hospital, London
Dr J I M Jones	Crookes Laboratory, London
Dr D Longson	Manchester Royal Infirmary
Dr F. Paulsen	Ferring, Malmo, Sweden
Dr J K Norymberski	Medical Research Council, Unit for the clinical pathology of steroids, Sheffield
Dr J G Phillips	University Dept. of Zoology, Sheffield
Dr E Ross	University College Hospital, London
Dr S Shuster	The Royal Infirmary, Cardiff.
Dr Bertha Singer	University Dept of Medicine, Sheffield
Dr. M. P Stack-Dunne	Medical Research Council, Hampstead, London
Dr J M Stowers	Queen's College, Dundee
Prof. T Symington	Royal Infirmary, Glasgow
Prof C H Stuart-Harris	University of Sheffield
Dr. W J Tindall	Organon, London
Dr H F West	Rheumatism Research Centre, Sheffield
Prof Graham Wilson	University of Sheffield

CONTENTS

THE NATURE OF CORTICOTROPINS AND THEIR RELATION TO MSH

H.B.F. DIXON
Dept. of Biochemistry University of Cambridge

Groups of workers who have isolated pig corticotropins (1, 2, 3) found several active components in concentrates of pituitary glands. Apart from recognizing that one component was formed by loss of amide ammonia from the main form of the hormone, they confined their attention to the component present in the greatest amount, and only its structure has been studied.

Fig. 1 shows the sequence found by Shepherd et al. (4) It has recently been confirmed by Harris & Waller (unpublished) when they used a new proteolytic enzyme to redetermine the sequence at loci 25–28. The sequence had proved particularly difficult to determine in this region and there was disagreement between different groups of workers

Corticotropins have also been isolated from sheep, ox and man The sheep hormone seems to differ from pig ACTH both in the region 25–28 and also in 31–32 where *Ala.Ser* replace *Leu Ala.* (5), while the ox hormone, again apparently different at loci 25–28 has *Ser Ala* at 31–32 (6). The amino acid analysis of the human hormone is identical with ox and sheep, though the sequence has not been fully determined (7) It thus contains 3 serine and 1 leucine residue whereas the pig hormone contains 2 of each.

1	2	3	4	5	6	7	8	9	10	11	12	13
H Ser	Tyr.	Ser	Met	Glu	His	Phe	Arg	Try	Gly.	Lys	Pro	Val
14	**15**	**16**	**17**	**18**	**19**	**20**	**21**	**22**	**23**	**24**	**25**	**26**
Gly	Lys	Lys	Arg	Arg	Pro	Val.	Lys.	Val	Tyr.	Pro	*Asp*	*Gly*
27	**28**	**29**	**30**	**31**	**32**	**33**	**34**	**35**	**36**	**37**	**38**	**39**
Ala	*Glu*	Asp	Glu	*Leu.*	*Ala*	Glu	Ala	Phe.	Pro	Leu	Glu	Phe OH

Fig 1 Sequence of pig corticotropin
The sequence appears to differ in the sheep and ox hormones at the loci underlined

Much of the interest of these structures lies more in the field of protein synthesis in general rather than in contributions to the study of corticotropin. The contribution of such chemical work to hormone physiology is partly because of the help provided in characterizing hormone preparations. If we look further ahead, structural work must contribute to knowledge of the mechanism of action, since the action must reflect the nature of the hormone.

One approach to problems of action lies in finding what parts of the structure are necessary for hormonal action, and here most progress has been made in the related field of melanocyte-stimulating hormone (MSH). Frogs and toads are able to darken their colour when they are in dark surroundings. Their pituitary glands secret MSH which aets on the melanocytes of their skin. When the skin darkens the melanin granules in the melanocytes spread from a compact bunch in the centre of the star-shaped cell until dispersed throughout all its extented processes

In most extractions, and in the oxycellulose concentration, MSH is concentrated with ACTH Thus oxycellulose concentrates of ACTH, often labelled 'highly purified corticotropin', are almost as highly purified MSH preparations MSH can, however, be separated from ACTH and two types have been isolated The structures of these substances (8, 9) are given in Figure 2. It is interesting that they share a common sequence with ACTH, which doubtless explains the slight MSH activity of highly purified corticotropins

Different types of assay for MSH activity give different relative potencies for the various substances This is to be expected when the activity measured is as complex as a hormonal one For the hormone to act it has probably to undergo a series of reactions and these may have different specificities There may, for example, be one reaction of binding the hormone to the cell it acts on, and another by which it exerts its action when bound The conditions of assay will determine which of the necessary steps is most rate-limiting, and hence different conditions of assay may produce different relative potencies for a pair of substances The relative potencies given in Figure 2 are therefore approximate.

From Fig 2 it can be seen that *the terminal serine residue of corticotropin* is outside the sequence possessed in common with MSHs and so is probably not necessary for MSH activity. To confirm this, two different attacks have been made on it. One was a specific acetylation of the free amino group of this serine residue (10) The resulting product

																13		39	Relative potency
ACTH				Ser	*Tyr.*	Ser.	*Met*	*Glu*	*His*	*Phe.*	*Arg.*	*Try.*	*Gly.*	Lys.	*Pro.*	Val	..	Phe.	1
α-MSH		CH_3	CO	Ser	*Tyr.*	Ser.	*Met*	*Glu*	*His*	*Phe.*	*Arg.*	*Try.*	*Gly.*	Lys.	*Pro.*	Val.	NH_2		250
β-MSH	Asp	Glu	Gly.	Pro	*Tyr.*	Lys.	*Met*	*Glu*	*His*	*Phe*	*Arg*	*Try*	*Gly*	Ser.	*Pro*	Pro	Lys.	Asp.	100

Fig 2 Pig hormones with MSH activity The common sequence is underlined

Ox					1 H-Asp.	2 Ser.	3 Gly	4 Pro	5 *Tyr*	6 Lys	7 *Met*	8 *Glu.*	9 *His.*	10 *Phe.*	11 *Arg.*	12 *Try*	13 *Gly.*	14 Ser.	15 *Pro.*	16 Pro.	17 Lys.	18 Asp.-OH
Pig					1 H-Asp	2 Glu	3 Gly.	4 Pro	5 *Tyr*	6 Lys	7 *Met*	8 *Glu*	9 *His*	10 *Phe.*	11 *Arg.*	12 *Try.*	13 *Gly*	14 Ser.	15 *Pro.*	16 Pro.	17 Lys	18 Asp.-OH
Human	1 H-Ala	2 Glu.	3 Lys	4 Lys	5 Asp	6 Glu.	7 Gly.	8 Pro	9 *Tyr.*	10 Arg.	11 *Met*	12 *Glu*	13 *His*	14 *Phe.*	15 *Arg.*	16 *Try.*	17 *Gly*	18 Ser.	19 *Pro*	20 Pro.	21 Lys.	22 Asp -OH

Fig 3 Species differences in β-MSH
Loci where species differences occur are outlined.
The sequence possessed in common with α-MSH and ACTH is underlined.

had greatly diminished ACTH activity but possessed MSH activity. It might have been expected that it would have greatly increased MSH activity, since the acetylated ACTH has the complete structure of alpha-MSH except for one hydrogen atom but has a long peptide chain in addition. Alpha-MSH is about 250 times as active on a weight basis or about 80 times as active on a molar basis, so an 80-fold potentiation might have been expected. This was not unreasonable since Anfinsen (11) quotes Boissonnas as finding that synthetic alpha-MSH is 70 times as active as the corresponding compound without the terminal acetyl group. In fact only a 5-fold potentiation was found, and under some conditions of assay no potentiation at all. Thus the chain of residues from positions 14 to 39 must diminish the potential MSH action of ACTH.

Another approach was to destroy the terminal serine residues with periodate (12). By using mild conditions (0.0025 M periodate for 30 sec.) the risk of unspecific oxidation is made small The reaction is stopped and the product modified by the addition of borohydride. The product has greatly diminished ACTH activity, and somewhat increased MSH activity. Once more a change outside the common sequence does not destroy MSH activity. Dr Frank Engel of Duke University, North Carolina, has been studying the fat-mobilizing activity of the periodate-borohydride treated ACTH and finds that this extra-adrenal action is only slightly diminished in comparison with ACTH Yet oxidation by peroxide, which probably only changes one atom in ACTH, completely destroys its ACTH, MSH and fat-mobilizing actions (13) and this reaction can be reversed with a regain of all the activities Thus the fat-mobilizing activity has in some respects great specificity, so it is interesting that the periodate-borohydride treated ACTH should retain it The MSHs do not possess fat-mobilizing activity

Although most work has been done with pig preparations, MSHs have also been isolated from ox (14) and man (15) and their structures (Fig. 3) have been determined (14, 16). The ox hormone differs from the pig hormone in the change of an amino acid at one locus The human hormone differs not only in a substitution of the same kind, but also in having four extra amino-acid residues at one end.

Until recently there had been speculation whether MSH had any function in mammals or whether it occurred in their pituitary glands simply as a residue from an earlier stage of evolution Since the most probable mutations that could affect the production of MSH would eliminate it completely, and other likely ones would affect a part of the common

structure that was necessary for activity, the view that MSH had no mammalian function depended on the belief that no mutation that affected its formation had occurred since mammals evolved. But inspection of the structures shows that profound mutations within mammals have affected MSH formation and so it is probable that many more have resulted in loss of the hormone Since their possessors have not survived, MSH must have survival value within mammals In other words that part of the structure which is necessary for activity has been preserved by natural selection, although the molecule has been changing. What function gives MSH its mammalian survival value is not known.

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DISCUSSION FOLLOWING DR. DIXON'S PAPER

Prof. Symington. (Opening discussion) I would like this to be quite informal—

Dr. Jones: I would like to ask Dr Dixon whether the alpha M S H with an ester group at one end and amino an group at the other end may, by any chance, be an artefact arising from the extraction of the ACTH from the pituitary with glacial acetic acid

Dr. Dixon: I don't think so because I have recently isolated it without using acetic acid—a hydrochloric acid acetone extraction, followed by extraction of the acetone with ether followed by desalting and with ion-exchange chromatography at the end I must admit that there was one exposure to acetic acid but there

had greatly diminished ACTH activity but possessed MSH activity. It might have been expected that it would have greatly increased MSH activity, since the acetylated ACTH has the complete structure of alpha-MSH except for one hydrogen atom but has a long peptide chain in addition. Alpha-MSH is about 250 times as active on a weight basis or about 85 times as active on a molar basis, so an 80-fold potentiation might have been expected. This was not unreasonable since Anfinsen (11) quotes Boissonnas as finding that synthetic alpha-MSH is 70 times as active as the corresponding compound without the terminal acetyl group. In fact only a 5-fold potentiation was found, and under some conditions of assay no potentiation at all. Thus the chain of residues from positions 14 to 39 must diminish the potential MSH action of ACTH.

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was not any hint of this happening to ACTH under similar conditions, but it was cold acetic acid and not the hot

Dr. Singer: . . . the hydrochloric acid? . .

Dr. Dixon: It would not acetylate . . .

Dr. Singer: No but would not the hydrochloric acid attack it in some way and convert it—a break down—?

Dr. Dixon: That is possible but I don't think it has happened It might be a degradation product of something bigger. The exposure to acetic acid was only for ½ hour with 50 % acetic acid at room temperature

Dr. Grant. I hope I will not be showing my ignorance too badly. You told us about a 39 amino acid polypeptide. That is the thing that you get from the oxycellulose columns. What sort of ACTH does one find in the usual commercial preparations that one injects into patients and uses experimentally on animals The Crookes and the Armour ACTH and so on How much do they differ from this 39 amino acid polypeptide.

Dr. Dixon: I believe that their main active component *is* that substance There are minor active components which differ in ways we don't know One of them merely by having an amide group missing, and there are almost certainly other substances adsorbed by the oxycellulose and so concentrated with the ACTH—, particularly beta M.S.H is the one we know about. We obviously have only been looking for things with recognisable activities So there are other pituitary substances in these preparations unless a step of high resolution has been used such as chromatography or counter—current distributions, which all the makers have done I understand, but not released for general use

Dr. Grant: As a steroid biochemist I find these ACTH's rather confusing I have had given me, from I think Organon, ACTH 'A' and ACTH 'B' and I have recently read a paper by Dick Savard in which he said, in America, that he used 'A' ACTH There is presumably as in so many similar fields no accepted nomenclature for various ACTH's, is there?

Dr. Dixon: Dr Stack-Dunne should answer that There has been a conference on standardization that came to some kind of agreement which was recommended to the World Health Organization

Dr. Grant: Has it been published?

Dr. Stack-Dunne: The conference proceedings have been circulated—there are copies available The actual recommendations on the corticotropin nomenclature have gone to the World Health Organization and are, I think, in the hands of the International Pharmacopeia We must wait to see what they make of the suggestions for nomenclature The World situation at the moment is that there is no general agreement on nomenclature If people say corticotropin 'A' without defining it as 'A_1', they probably mean a high potency or 'oxycellulose' corticotropin That is what they generally mean whereas corticotropin 'B' would be crude corticotropin, but if we say 'B' we are using the nomenclature of Fisher & White—the corticotropin has been reduced by actual degradation In that case, once more, the subcutaneous to intravenous potency would be reduced but for a different reason (When crude corticotropin is oxycellulose purified its subcutaneous potency is increased 2-3 times relative to the intravenous potency) In the normal type B' preparation it is probably the impurities that are responsible In pepsin degraded corticotropin, which we call corticotropin 'B', it is probably because of the degradation itself

Dr. Tindall: Actually the 'A', that you had is a chromatographically pure fraction and the 'B' ACTH that you had is in fact a pepsin degraded one

Dr. West: I think that we also mentioned corticotropin C which was to

be the name for chemically modified corticotropin I wonder whether Dr Dixon can tell us whether there are any chemically modified corticotropins other than those that have been hydrolyzed and that we call 'B'

Dr. Dixon: Was not the suggestion, when that was mentioned, that acid hydrolysis does degrade and still leave a thing that is active in certain ways I don't myself remember whether we persisted with that recommendation I have never myself handled such material

Dr. Fotherby: Is it not possible to get an octa-peptide with ACTH activity? Is this a product of hydrolysis or do you find it naturally occurring?

Dr. Dixon: I am afraid that this was a mistake Long before ACTH was pure there was a much larger molecule 'almost pure' that was thought to be ACTH It had, in fact, ACTH as a minor contaminant, 1 % or so, less than the limits of our criteria of protein purity of those days It could be hydrolysed down to an *average* size of 8 amino acids and there was then physical evidence that there was very little big stuff in it and it was still active But the point is the "very little big stuff in it" was this 1 % that was 39 amino acids and had come down to the order of 26—it explained its activity. A lot of work was based on the hope that something as small as 8 would be active

Dr. Chalmers: I wonder, Dr Dixon, whether you know whether M S H has any lipolytic activity in vitro

Dr. Dixon: Dr Engel found none

Dr. Singer: I wonder whether it has ever been detected in blood M S H?

Dr. Dixon: Yes

Dr. Singer: Has it been detected in Addison's disease?

Dr. Dixon: I believe so

Dr. Singer: Do you think that that might explain the pigmentation?

Dr. Dixon: I believe that it might But there is the danger here Is it the M S H activity of ACTH or is it M S H? I gather the amounts found in Addison's disease—but I have not checked this myself—of M S H activity are a bit high to be due to ACTH So probably there is increased M S H secretion

Dr. Beryl Davies: After the oxycellulose extraction, how do you then separate ACTH and M S H?

Dr. Dixon. Different chromatographic systems The ACTH, *we* separate, on carboxylic resins The beta M S H is unretarded and is then chromatographed on a sulphonic resin The alpha M S H cannot be recovered from that procedure but *some* can be got separate, not a quantitative yield, at an *earlier* stage when ACTH and much beta M S H are precipitated by raising the acetone content The alpha M S H stays in the acetone supernatant and can be concentrated by fairly unspecific methods Finally the alpha-M S H is itself chromatographed on a sulphonic resin The sulphonic resin chromatographies for alpha and beta—M S H are rather alkaline conditions about pH 10 As they are very basic substances they stick to the resins firmly and the alpha—M S H will only come out of the resin if you add some urea

Dr. West: The corticotropins in common use, the C O C purified corticotropins, have you any idea how much of these preparations does not consist of corticotropins A_{1-2-3}? That perhaps one may call impurity?

Dr. Jones: About 50 % I would say Judging by the activity recovered

Prof. Graham Wilson: If you have used corticotropin for a long period in treating a disease, do you actually get pigmentation? I don't think you do Do you?

Dr. Holti: I have seen a fairly large number of patients treated with ACTH, in my limited experience, and three patients who had white hair before treatment

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Dr. Dixon: I believe that it might But there is the danger here Is it the M S H activity of ACTH or is it M S H? I gather the amounts found in Addison's disease—but I have not checked this myself—of M S H activity are a bit high to be due to ACTH So probably there is increased M S H secretion

Dr. Beryl Davies: After the oxycellulose extraction, how do you then separate ACTH and M S H?

Dr. Dixon: Different chromatographic systems The ACTH, *we* separate, on carboxylic resins The beta M S H is unretarded and is then chromatographed on a sulphonic resin The alpha M S H cannot be recovered from that procedure but *some* can be got separate, not a quantitative yield, at an *earlier* stage when ACTH and much beta M S H are precipitated by raising the acetone content The alpha M S H stays in the acetone supernatant and can be concentrated by fairly unspecific methods Finally the alpha-M S H is itself chromatographed on a sulphonic resin The sulphonic resin chromatographies for alpha and beta—M S H are rather alkaline conditions about pH 10 As they are very basic substances they stick to the resins firmly and the alpha—M S H will only come out of the resin if you add some urea

Dr. West: The corticotropins in common use, the C O C purified corticotropins, have you any idea how much of these preparations does not consist of corticotropins A_{1-2-3}? That perhaps one may call impurity?

Dr. Jones: About 50 % I would say Judging by the activity recovered.

Prof. Graham Wilson: If you have used corticotropin for a long period in treating a disease, do you actually get pigmentation? I don't think you do Do you?

Dr. Holti: I have seen a fairly large number of patients treated with ACTH, in my limited experience, and three patients who had white hair before treatment

started, definitely grew black hair while they were being treated with ACTH, but as soon as treatment was stopped their hair again turned white I wrote to Armours about this in 1952 and they said that they were quite aware of this fact and that they had observed it repeatedly in the States It is still a fairly rare occurrence

Dr. Dixon: I think that that will almost certainly be due to the M S H content of the oxycellulose preparations This is a difficult point—of what M S H. does—because in the amphibian the obvious effect is the quick, within half an hour, dispersal of melanin granules, but there is also evidence that in amphibia you do get, on prolonged treatment, an increase in total melanin It not only has the short effect but it also stimulates melanin production In man the only well authenticated effect is the increase in melanin but so far it has been done only with rather crude M S H. preparations which may owe their activity partly to ACTH or to degraded ACTH Probably the doses used in treatment are too small to get much of this effect

Dr. Cates: I think that we are confusing the colour of the hair with the colour of the skin, aren't we? If we start talking about M.S H making peoples hair black when it was white? It is not the same process at all, is it?

Dr. Holti: Well melanin is produced, of course, by melanocytes and whether it subsequently appears in skin or hair the underlying process is identical.

Dr. Cates: Clinically it is not Patients who have Addisons *disease do not* get appreciable change in the colour of the hair whereas they do get appreciable change in the colour of their skin Whereas people with Cushing's disease get changes in the colour of their hair without changes in the colour of their skin I think that we are begging lots of questions if we say that the change of hair colour is evidence of ACTH

Dr. Cope: There are some clinical problems here that may resolve later. I don't ever recall seeing pigmentation occur under ACTH therapy but others have had much more experience of long-term ACTH than I have. Whereas in people who might develop M S H 's or ACTH's (?) of their own endogenous type, you may get intense pigmentation, not only in Addison's disease but after Cushing's syndrome when a sub-total adrenalectomy has been done We have had one recently where the man was actually almost negroid, with skin pigmentation, with no evidence of hyperactivity of his adrenal at all. Presumably there was *ACTH plus a melanophore stimulating hormone production of a high degree in him.*

Dr. Dixon: I think I may have been overstating the case of the oxycellulose concentrates. They are quite good concentrates of M S H but you do lose a lot of M S H in making an oxycellulose concentrate of ACTH, so the treatment with ACTH is not as strong a treatment with M S H as you could have given

Mr. Forrest: But you surely pointed out that the ox M S H. is different from the human and you are treating people with ox and pig ACTH

Dr. Dixon: Pig M S H has been shown to be active in humans, a *crude* pig M S H.

Mr. Forrest: In what way was this shown

Dr. Dixon: *By increased pigmentation of the skin*

Dr. Davis: We have certainly seen generalized pigmentation occur with long term corticotropin in a few cases, probably 3–4 %, something of that order We thought at one time that it might have something to do with different batches but came to the conclusion hat it was an individual susceptibility There were some people who became pigmented when others did not. It was not due to particularly high doses—as measured by 17(OH)CS excretion

Dr. Cope: Were they the dark skinned people?

Dr. Davis: They were—the underlyingly dark skinned

Dr. Shuster: There is a photograph in a paper by Lerner of a negress who got very much darker after corticotropin therapy I would like to ask whether procedures affecting corticotropin *release* make any difference to blood or urine M.S H. excretion

Dr. Dixon: I believe that corticosteroid treatment, Lerner has shown, has lowered M.S H. when it was high. I think that M S H is hard to find in the normal It is in cases like Addison's disease that you can find it

Voice: Stressed rats for example have an increased M S H in the urine

Dr. West: I think that I can confirm what Dr Dixon was saying about the effect of M.S.H In the old days when we used crude ACTH we saw a lot of pigmentation and one or two dark haired patients actually went mahogany—they were having fairly high doses Today's patients have received only C O C. purified corticotropin for 5–6 years and the only ones that are pigmented, they are not grossly pigmented, the only ones that are definitely pigmented became pigmented on the crude ACTH. It may be, as you say, that there has been an actual increase in the melanophore producing cells that has not disappeared, so that they have remained darker I have not seen any patients who have never had crude, pre-C O C purified, corticotropin become pigmented in the doses we normally use

Mr. Forrest: There must have been a lot of hypophysectomies done in the States now on negroes. I have not seen any reports of any of them becoming paler I have two patients who have been hypophysectomized, both of the darker skin type, and both of them complain that when they got out into the sun, whereas they used to become brown now they become pink and sunburn easily. This is an isolated observation, I have not seen it in any others In those two patients it was quite a striking complaint though It may be that the pituitaries taken from negroes have a different M S H. content or a different M S H from white people Is this a possible mutation that has taken place within the human race?

Dr. Dixon: It is just possible but I should be very surprised The kind of thing is that in a disease of the skin—I think the evidence is some of Lerner's—where you got de-pigmented patches in some negroes—these were completely unresponsive to M.S H M S H treatment which he hoped might restore the pigment in fact made the appearance of the person worse by making darker the pigmented parts.

Dr. James: How many different ACTH fractions do you get from fractionating the crude mixture?

Dr. Dixon: One separation about 4 or 5, another separation about the same—yes 4 or 5.

Dr. James: Do they have different biological activities?

Dr. Dixon: No they seem to be mg for mg much the same

Dr. Singer: Steroidogenic or ascorbic acid depletion?

Dr. Dixon: Ascorbic acid depletion

Dr. James: What about the production of steroids?

Dr. West: What we have done is to compare A 1 & A 2 Mg for mg. they were equally steroidogenic in me I wonder whether Dr Dixon could tell us anything to help with the antigenicity studies I believe that a great deal is know about the structure of various insulins and a great deal is known about their antigenic properties I wondered whether one could draw a parallel from insulin to corticotropins and their species differences Whether you could suggest how they might or might not have antigenic differences

Dr. Dixon: I think the sheep and the ox ACTH are slightly closer to human than the pig Whether any of the differences are large enough to be identifiable with an antigen-antibody reaction I don't know. A partial structure of human *ACTH* has just been published by Lerner and co-workers a few weeks ago and it does seem to be more like the sheep and ox, whose complete structures are not known, but there is one amino acid difference as well as difference in sequence. So it just might be that if you did get a person reacting to pig that sheep or ox highly purified might prove better

Dr. Beryl Davies: Dr Currie and I have been using pure pig A_1 (Organon) and trying to see whether it is antigenic in rabbits. In a couple of rabbits we have produced evidence of anti-hormone activity; but this is not linked with any precipitating antibody activity Whether this is because the antigen used is a pure polypeptide we don't know One other factor was that in the original experiment on two rabbits we used the pure pig ACTH after it had been treated with potassium alum We now find that most of the activity is not precipitated by the potassium alum So in current experiments we are just using A_1 dissolved in saline as our antigen rather than potassium alum precipitated material Our other observation is on the storage form of ACTH in human pituitaries We think there that some of the material is protein bound and some of it is free polypeptide, in that by treating human pituitaries with 2 5 % trichloracetic acid you get from 50 % to a negligible amount of ACTH in your solution Perhaps this ties in with the observation that alpha and beta corticotropins are soluble in trichloracetic acid—this form is coming out into the trichloracetic acid

Dr. West: I did not quite catch what you said about the protein binding

Dr. Beryl Davies: These were human pituitaries and they were treated with 2 5 % trichloracetic acid—stirred in this Some biological activity is detectable in the trichloracetic acid solution—as alpha and beta *corticotropin* are said to be soluble in trichloracetic acid one would think that this is what one was removing from the human pituitaries; but as one does not get the total content of the pituitary out into solution one would think that what does not come out of the gland into trichloracetic acid is protein bound

Dr. Dixon: I do have some evidence, but I have not done activity determinations yet, that there are at least 4 and possibly 5 forms of human ACTH The nasty thing about them is that, unlike pig, *one* does not predominate So we get horribly small amounts—a little of everything

Dr. Beryl Davies: That might explain the discrepancies in amount that you get in trichloracetic when you repeat it with different samples of human pituitary

Dr. West: Dr Dixon, hasn't Professor Young spoken of a proto-corticotropin—a *precursor* of corticotropin? Pre-corticotropin?

Dr. Dixon: Yes The evidence that Dasgupta got was this, that you can get some activity out of extracts of pituitaries of this kind—that injected in an ascorbic acid depletion test by *itself*, at a certain dilution, it is inactive Treated very very mildly with acid, just bring to pH3 and then back to neutral, or treated with urea, and then injected—it is active That is the evidence Whether it is an artefact or whether it really reflects some physiological activation of corticotropin we don't know It is a very small part of the activity—it is got actually by centrifuging down the particles from a pituitary homogenate. They carry most of the activity and leave a supernatant that is inactive at certain dilutions unless this treatment is done. So it is a kind of denaturing condition which releases activity in this case

Prof. Symington: Thank you very much Dr Dixon for a very interesting communication. I will now ask Dr Stack-Dunne to speak on the biological assay of corticotropins.

THE BIOASSAY OF CORTICOTROPINS

M. P. Stack-Dunne
Medical Research Council laboratories Hampstead London

The instructions given me were that I should outline the present situation in the bioassay of the corticotropins. I am going to take corticotropins to mean the adrenal stimulating preparation, very probably a mixture of active polypeptides, typically prepared by oxycellulose purification of pituitary extracts.

For the standardization of this material for clinical use, the only reliable and practical assay methods are in my opinion those based on ascorbic acid depletion in the rat adrenal, using subcutaneous injection where the material is to be used non-intravenously, or following intravenous injection where that is to be the route of administration to the patient.

This was discussed at length at the International Conference on Corticotropin Standardization, London, 1957. If anyone has doubts about the validity of this statement perhaps they can be raised in the discussion.

I suppose the refinement of bioassay methods, where these are unlikely to affect materially the quality or potency of the corticotropin as it reaches the clinician, is not of immediate interest to this audience, but I would like to mention some excellent work in this direction by Rerup, published in Acta Endocrinologica Volumes 25, 28 and 29 He has re-examined the standard methods and suggested improvements in procedure and statistical design. Guillemin and his collaborators (Endocrinology Vol 60, 63 & 65) have shown how considerable improvements in sensitivity and precision can be obtained. I would also like to remind you of the interesting observation of Dasgupta and Young of a fraction in certain types of pituitary extracts, which can be activated by mild acid treatment or exposure to urea, the activity measured by the intravenous Sayers assay It is unlikely that this material will be present in oxycellulose purified corticotropin, but if it is not an artefact of isolation, it may be of significance when corticotropin in body fluids is under investigation

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The aspects of corticotropin standardization I am going to dwell on more thoroughly are those related to the degree of satisfaction the present control methods arouse in the clinicians responsible for using corticotropin in their patients. In this country, during the past few years, there has certainly been some serious confusion, but this should have, and probably has, largely been eliminated by the introduction of the exclusive use of high potency corticotropin. However, a possible source of irregularities in the standardization of clinical corticotropin remains in the multiplicity of standards used in its control. This has come about through the wish to ensure that clinical corticotropin be standardized against material of similar chemical and biological type It has been necessary, while waiting for the introduction of the Third International Standard, (which, *unlike* the *present* Standard, will be made from oxycellulose—purified corticotropin) for the manufacturers to establish individual house standards Technically, it is a very difficult matter mutually to adjust a series of standards to a sufficient degree of accuracy and we will all feel much more secure when the new standard is available. It is hoped that an International working Standard, prepared in a way identical to that of the International Standard and from the same batch of material, will be adopted for daily use by all manufacturing laboratories. This innovation should, with a little good fortune—and there are many uncertainties in the bioassay of corticotropin—put the control position finally in order

A matter of some concern is the stability of corticotropin preparations. It may not be generally realised that the 16 % gelatin preparations, if they are to keep their potency until the stated expiry date, must be stored in a refrigerator In the past, as discussed at the International Conference in 1957, there have been isolated batches with very low stability, and this led to considerable confusion. This was partly responsible for sparking off criticism of the basic assay procedures I know of no recent occurences of this sort, but it is a point we ourselves are watching continuously. Accelerated degradation tests are run on all batches issued in this country.

To summarise the control situation: (a) I feel reasonably confident that we are using assay methods relevant to the clinical use of corticotropin, and that the considerable care with which the manufacturers apply these methods should assure that within one manufacturers' product no clinically significant variation in potency occurs (b) The position between manufacturers is not so clear, but the advent of the third standard can

be expected to remedy any discrepancies that exist. (c) *In relation to* the possibility of undesirable side-effects or tendency to cause resistance, I know of no clinical observations at present, which suggest useful modification of the present control methods or relevant laboratory investigations. The technical problems that will still remain are more significant to the increasingly accurate measurement of activities demanded, probably, mainly for commercial reasons, than to the clinical use of the material. But, if there *is* a serious flaw in the control situation, it is that it is so difficult to get clinical data of sufficient weight to check whether or not the standardization of corticotropin is really doing all that it should for the clinician, and I would like to consider this last point *in more* detail

In the few experiments where corticotropin has been assayed in human *subjects with an adequate experimental design, an index of precision* of 0.2–0 3 has been obtained, that is, about the same as with rats in the Sayers assay. This means in practice that to be able to assert that a particular batch of corticotropin was one half or twice the potency that it should be, a number of human observations roughly equal to the number of rats used in the original bio-assay would be needed, that is, 50 or more. Larger discrepancies would, of course, be significant with fewer observations. These estimates of the index of precision were based on healthy, carefully selected human subjects If assay precision deteriorates with sickness, in humans in at all the way it does in rats, I suspect that only very extreme discrepancies will be significantly revealed in normal clinical experience. In a prolonged course of treatment of one patient, better discrimination may be obtainable, but particular care will be needed to ensure objectivity in this situation Unless an arbitrary programme of materials and dosages is drawn up at the outset, changes will tend to be suggested by changes in the patient's symptoms, just when his sensitivity is most likely to be altering Moreover, I have the impression from the published *reports that slow cyclic changes in patient sensitivity*, as measured by, for example, the excretion of 17-hydroxycorticosteroids, are *quite common so that there may be serious loss in precision* by extending the observations over several weeks

Because the sick individual is unlikely to be a very reliable bioassay animal, and because it is precisely from *his* biological response that we are to judge whether or not an adequate level of standardization has been achieved, it is necessary to consider the pooled data from many patients If in the future it is the opinion of clinicians that the control

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Dr. Jones: One injection

Dr. Stack-Dunne: The way that the hydrocortisone method, as suggested by Dr Tindall, is used, is to give three injections of the corticotropin at one hour intervals, hour 0, hour 1, hour 2 and killed at hour 3 I think that where you are comparing crude with pure corticotropin, and we have some experimental evidence of this kind of effect, the different injection routines may give rise to apparent differences in the relative potency Of course the hydrocortisone prepared animal should be sensitive to C R F. (the corticotropin releasing factor) although this is probably not present in the purified pituitary preparations This also might cause apparent differences in potency. Would that be likely Dr Tindall?

Dr. Tindall: I don't think that it would be likely to happen but it could do

Dr. Singer: I cannot see how you can expect to use the clinical material to compare with your standard assay in animals, because your animals are used once and they have not evolved any resistance The human is more complicated and may, after long term treatment develope a resistance

Dr. Stack-Dunne: I think that is correct

Dr. Singer: Therefore your standardization of ACTH in animals will be perfectly valid but it will not be the same as in a human being after repeated injections

Dr. Stack-Dunne: I see, yes, your points are valid, but the question is begged, may be, by saying that your standardization in rats is perfectly valid If you mean valid in a self contained sense in that experiment, yes, it certainly is I suppose, but is it valid as an assay for the standardization of corticotropin for the use in patients Now some clinicians would say that they had experienced difficulties in treating patients which was attributable to the fact that the bioassay methods were invalid for their use

Dr. Singer: I have a feeling that every subject who has had ACTH requires his own standardization

Dr. Stack-Dunne: If that is so we cannot standardize for each patient But we do want to standardize the corticotropin so that the variations in patients are not significantly due to the variations in corticotropin We want to achieve a level of control so that the doctor can rest secure in a reliable product, and his only problems be in the patient

Dr. Davis: Surely we have missed a point in the assessment You have said that we would need as many or more patients than rats—overlooking the fact that we are doing cross-over experiments using the patients as their own controls When we have made complaints about standardization it has been based on that kind of evidence

Dr. Stack-Dunne: There are some reports in the literature, I think that it was Dr Forsham's group, where they did an accurate bioassay on two preparations on normal healthy male subjects, using a very carefully designed cross-over technique, as in bioassays, and their index of precision on two estimates were 0 16 and 0 28, I think, something of that order It would seem to me from the published indices of precision that the amount of information in one human is not much more that in one rat Which means that if you want to give a degree of precision, with a reasonable degree of certainty, the clinician is subject to the same statistical limitations as the bioassayist

Dr. Davis: Yes That was done on the short term basis I was not very happy, using long acting preparations, about the 17(OH)CS excretion pattern in the first few days of treatment, we observed the peaking up and then the coming down I was thinking of the A.C O C comparison we did It averaged out all right but there were some discrepancies

methods need further refinement it is very desirable that they organise some method for collecting the independent observations of a large number of clinicians on a particular type of product or a particular batch. It is conceivable that this could be organised by those not actually using corticotropin, but it seems likely that the clinically important issues are more certain to be considered if the movement comes from the clinicians themselves.

Manufacturers, clinicians and control departments are all very much concerned that biological standardization should fulfil its purpose, and in the case of corticotropin, probably we all have some doubts as to whether this is so. This may be because we have not always fully exploited the information potentially available, and perhaps the most important advances in standardization will come from rather obvious improvements in communication

DISCUSSION FOLLOWING DR STACK-DUNNE'S PAPER

Dr. Jones: It is the standard practice among clinicians to assess the activity of corticotropin preparations by determination of corticoids excreted in urine One would therefore think that the best method of assay would be one in which a similar criterion was used for measurement Well this has been used in in-vivo experiments by the analysis of guinea-pig urine and by in-vitro experiments by the method of Saffran & Schally in which slices of adrenal tissue are incubated with ACTH and the corticoids liberated estimated In my experience neither of these methods give as satisfactory results as the ascorbic acid depletion method. I wonder what are Dr Stack-Dunne's experiences.

Dr. Stack-Dunne. Yes The in-vitro method is quite easy to do and the precision is high but we have found—everyone that has reported on it has found—that it correlates far more closely with the intravenous injection method in the Sayer's assay— It is not reliable since most of the corticotropin used is given intramuscularly and so Sayer's assay must be done by the subcutaneous method With the guinea-pig excretion—we have also got an assay going of that sort—where we have tested it, it has run parallel to the subcutaneous Sayer's assay but it is much less precise and there is a great deal more work to be done on it So far we have found no discrepancy between the subcutaneous and the guinea-pig 17-hydroxy excretion method So we see no reason to change over to it at the moment Its chief trouble is that it is much less precise

Dr. Jones: Yes That is my experience too. There is another point In the Sayer's assay the usual procedure is to hypophysectomize the rats but the suppression of the pituitary by the injection of hydrocortisone is also used I believe that Dr Tindall has used that method I have recently compared the two in about 18 parallel assays and I find that there is perhaps a just significantly higher value obtained by the hydrocortisone method than by the hypophysectomy method—to the extent of about 7–10 %

Dr. Stack-Dunne: May I ask a question? Was that using three injections or one injection, in the hydrocortisone method?

the dose. That is in rats What we have done is to carry out assays intravenously and subcutaneously using a large range of doses so that we can assess the dose required to produce the same depletion of ascorbic acid It is between 50 & 70

Dr. Cope: Dr Stack-Dunne's main plea is really for a better feed-back from the clinicians If one tries to analyse the consumer end a bit the uses to which corticotropin is put are highly heterogeneous It is very difficult to get comparable series of cases of any condition at all except, I suggest, in rheumatoid arthritis I think that it is almost unique among all the situations where corticotropin is used, where there is an approach to relative homogeneity of the type of material used

Dr. Stack-Dunne: May I ask about the treatment of diseases of the eyes? There is a reasonably normal patient to deal with and the symptoms are usually completely under control.

Dr. Cope: I was thinking of the general wards actually What I was going to suggest was that if you want the feed-back it should be organised either among the rheumatoid arthritis group, which is well represented here, or among the normal healthy population Now that could be arranged quite easily in almost any centre and I should have thought should become a routine testing methods for all corticotropin—or at least of reference batches that are intended for use on human material. It has been done before I think that it was done with B_{12} before it was characterized

Dr. Stack-Dunne: I am not quite sure that I quite follow you I was thinking of a feed-back of information on the forms of corticotropin as it is normally used.

Dr. Cope: I very much doubt whether you would get useful feed-back from what I have called the heterogenous use Now other clinicians may be able to speak on this We have on several occasions thought that a batch was inactive and on consulting our colleagues, who were using the same batch in the hospital, we were able to get evidence from them that it was us or the patient and not the batch that was inactive Now that sort of thing would have to be controlled or you would get a very heterogeneous collection of feed-back material

Dr. Stack-Dunne: Yes, if it is heterogeneous and only a single individual thinks the batch is inactive while everyone else thinks that it is all right, or makes no comment on it, then you don't have to concern yourself If some people or all your informers start saying it's wrong, it's wrong, it's wrong, (!) then you say there really is something worth looking at Because to do the biological assay to check the potency of one batch is a great deal of work—a very expensive procedure With scores of batches going through all the time you want to know which one you should look at and we don't know which one is worth looking at so we let them all go through If there was a sudden outbreak of complaints about one batch we would then know that there is a batch which may have gone wrong or for which the biological assay was misleading

Dr. Cope: Is it not then too late usually?

Dr. Stack-Dunne: It is then usually too late but one might gain valuable experience to stop it happening again, if it ever happens

Dr. Tindall One of the difficulties I think is that most of the clinical doses given are scarcely above threshold There is the possibility of a patient being unresponsive to these doses Most of the doses given in clinical practice are very low When we come to examine the results of giving higher doses the results are quite interesting Showing quite different caracteristics for different preparations and different doses

Dr. Stack-Dunne: There is the psychological point. When one is doing biological *experiments in experimental animals you always, during the course of a year,* get two or three things which look very interesting and very obscure, but when they are not hypothesised at the start and when they are very odd they usually turn out to be a fluke or accident. One finds that one just has to accumulate—to be really sure whether it is really true—a number of observations The point I was trying to make is that if a batch is suspected of being wrong, it would be very very useful if a lot of physicians who might have noticed this could all say so at the same time

Dr. Davis: Of course there have been big differences and that in fact happened when we did have those batches going off There was a sixfold difference (between the labelled potency and that found at re-assay 1954) But even with the less marked differences, I think that at that conference we would get up and agree that certain batches were bad—Dr West and ourselves and Professor Prunty.

Dr. Stack-Dunne: I feel a little unhappy about that kind of agreement, it is not independent, you did have a chance to talk to one another.

Dr. Davis: Only in public.

Dr. West: I think that one of the snags here is that when you assay corticotropin in rats you use a lyophilized preparation and when *we* assay it it has been, in some cases, interfered with en route Armours for instance talk about 'process control', what that is they do no say, it is put in some form of long acting delaying menstruum—then *we* assay it One of the examples which was quoted, some years ago, was a particular batch of Armours labelled 80,001. This turned out to be about three times as potent as any other batch and we referred it back to Armours and they re-assayed it and the assays were unchanged Possibly something had happened to that before we got it that made it extremely potent So it may be that our assays may not correspond with yours

Dr. Stack-Dunne: This is the kind of information There is no reason why this should not be so It may well be If these batches do show high potency that is not revealed by the Sayer's subcutaneous assay This is precisely the kind of thing that we want to be able to test

Dr. Davis: (Showing slide) That is batch 80,001 showing the differences we were getting P36102 was a reasonably standard batch 80,001 was the peak batch that Dr West was talking about

Dr. Stack-Dunne: Will that batch all be used up now?

Dr. Davis: We saved a little bit but last time we tried it on a patient it had gone off

Dr. Dixon: May I ask whether there is any more information on why there is this vast discrepancy between the subcutaneous and intravenous routes The explanations proposed are that something which delays absorption into the body will potentiate a thing if given subcutaneously and that with very crude materials if there is a proteolytic enzyme present it will lower the subcutaneous potency I have the impression that there are unpredictable changes between these two

Dr. Stack-Dunne: Since there is up to 50 % of impurity in clinical corticotropin the situation could obviously be very complex

Dr. Jones: Finally the product is assayed against a standard of the same kind and we get the same results, by the intravenous and the subcutaneous assay

Dr. Dixon: That is very interesting because some years ago certain producers were not saying that They said that they got different ratios.

Dr. Jones: Another point is that to produce the same physiological effect by subcutaneous injection and by intravenous injection you need round about 50 times

Dr. Singer: Is the effect of ACTH on the production of steroids due to a different chemical action of ACTH from that which is responsible for the change in adrenal size and histology In other words, does ACTH have two different chemical actions?

Dr. Grant: We would wonder about this We have a feeling, although we have not proved it, that there is an increase in activity or an increase in amount of some of the enzyme systems responsible for cortico-steroid biosynthesis in the slow long term action of ACTH We could not account for the sudden spurt of hydrocortisone secretion that you got within an hour of giving an intravenous injection of ACTH

Prof. Symington: There is more to be said of morphological and enzyme changes in the adrenal this afternoon Could we defer this discussion to that part of the programme this afternoon

Dr. Tindall: While we are on the chemistry may I just say that Dr. Wilmer Homan believes on the physical evidence that the fraction A_4 on the chromatogram is fairly powerfully a growth factor for the adrenal, and is in fact very weak as a steroid producer

Dr. Stack-Dunne: May I ask is that in the rat?

Dr. Tindall: Yes. It has not been tried on humans

Dr. Stack-Dunne: It is rather a toxic fraction I think

Dr. Dixon: I think this point of different chemical actions, if this subject is permissible, is a very interesting one For example what I said about different M.S H actions—results of assays—under slightly different conditions, suggests that these hormones have to undergo one or two reactions which may have slightly different specificities Now Dr Engel believes that two specificities of ACTH may be (1) the action and (2) the binding to the adrenal Some of his evidence is that if you oxidize the ACTH it loses its activity completely in the adrenal and on adipose tissue If you reduce it back both activities return So the fat pad has some specificity, for it is only the introduction of one oxygen into the molecule we believe, yet the periodate treated corticotropin that I sent him is almost fully active on the adipose tissue but has lost most, all but 1 or 2 % of its activity, on the adrenal Now he interprets that as meaning that this ACTH is still fully active but has lost its specificity of binding to the adrenal tissue Well there are a lot of speculative steps in that but is does suggest that different chemical actions of ACTH could exist

Dr. Singer: There is one point on the Saffran assay that Dr Grant has not mentioned Dr Roberts has presented evidence that the rat adrenal in-vitro is much more sensitive to rat ACTH than to the other ACTH preparations if they are assayed by the Sayer's assay

Dr. Stack-Dunne: In all complete biological systems there seem to be very many stages each possibly influenced by the chemical nature of, in this case, a hormone, and the nature of accompanying impurities The 'stages' I am thinking of, are absorption from an injection site, transport to the target organs, and action at the target, with possibilities of differences in the time relationships, and in the modification or destruction of the biological activity at each stage Logically, one would expect any differences in the system due to variations *in the hormone-impurity* mixture injected to produce some differences in the biological effect One feels the differences in effect will certainly be there Their magnitude is the difficult point

Dr. Dixon: Dr Fortier has evidence that the rate of binding of rat and pig ACTH to the rat adrenal is very different

Dr. Hemingway: Just with reference to the first part of the question of cutting the adrenals We have had some evidence both from tissue damage and from

Dr. Grant: I would like to make two points The first rather a minor one that you can dispose of quickly. Last year we had Dr Murray Saffran in Edinburgh and had the opportunity of benefitting by his experience in the in-vitro assay. Some very interesting things arose A man in the biochemistry department in Glasgow was trying to do the Saffran assay and was consistently getting low results. When he came across to Saffran to ask him why, Saffran asked him how he was dividing the rats adrenals when he cut them in two He was cutting them with a scalpel and Saffran said "no you must not cut them with a scalpel, you must cut them with a sharp pair of scissors". When he went back and cut them with sharp scissors the assay went up beautifully. Now we were doing the same sort of thing in the biochemistry department in Edinburgh at that time, we were not using a scalpel we were using very sharp Wilkinson razor blades We talked to Dr Martha Vogt about this because she had a wide experience of the output of the rat adrenal in-vivo and she said "Yes, if you traumatize that adrenal, if you cause any congestion even for a short time when you are cannulating the renal vein, to get adrenal venous blood, you will get a fall in the output of adrenal steroids of that rat's adrenal". I think that people should realize that although, once you have got it going *well* the Saffran Schally assay is a very nice thing, there are big snags in it. The second point I would like to make, we would rather like information about this (Professor Symington is going to mention this this afternoon), is that we must distinguish two fundamental actions of ACTH There is the short term action and the long term action Now in your assay proceduce, the Saffran assay, you are measuring the short term thing The thing that Bush showed—a rise in an hour, or even less, after a shot of corticotropin. Then there is the effect you get in the prolonged administration of corticotropin Isn't this just the explanation of the difference between the intravenous administration and the subcutaneous or intramuscular? I would like to know somebody's opinion on this

Dr. Cates: Is there not another difference that may tie up with this? I don't know whether this is now 8 years out of date but when I was working in this field I believed that there were two separate actions of ACTH or two different ACTH's one of which was truly trophic There was Rinfret in California who was showing that he could get a substance that increased the adrenal weight only Now is that all supplanted nowadays because clinically it has a very important place in people who are on steroids and you want to see that they do not get adrenal atrophy

Dr. Stack-Dunne: These experimental observations are a little clearer now One of the clear cut things that come from the adrenal weight experiments that we were doing was that in the rat, the hypophysectomized rat, you can enormously synergise the effect of corticotropin on adrenal weight by giving growth hormone Down to doses as small as 25 microgrammes there is a significant effect You can increase the final size of the corticotropin treated hypophysectomized rat adrenal twofold or threefold But this is unlikely to be of *importance in* the human because pig and beef growth hormones are not active in humans and in any case the method is not applicable to a patient The observation of Rinfret was on the equine pituitary It has not been finally explained, but could have been due to growth hormone, he was working on the rat of course I think this preparation that Dasgupta called pre-corticotropin is active in the long term [illegible] vas active in the long [illegible] —although it was not [illegible] That means that pre-corticotropin was inactive in the intravenous test but seemed to be a natural *kind of* delayed preparation Slowly released in-vivo—*I* think that was *Dr Dasgupta's* opinion.

preparations. We ourselves have not noticed it going off while we have been using it We need some more information from you on the subject.

Dr. Stack-Dunne: We were not aware of this ourselves but we have been doing some accelerated degradation tests and the manufacturers now have also done some tests, and we know that preparations in 16 % gelatin have a known and measurable rate of decay The expiry date is 2 years after the date of manufacture. If they are kept in a cool place the fall in activity by the expiry date will not be significant It is much wiser to keep them in the refrigerator and in the new preparations this will be so marked I think that one manufacturer puts on the box—"keep in a cool place"

Dr. Singer: Yes, they say 50° F and then you find that they have not been kept in a refrigerator. I was told by our pharmacy that their room temperature was low enough—but in our laboratory the temperature is well over 50° F.

Dr. Stack-Dunne: If you are keeping corticotropin for more than six months it will be very important to see that it is correct

Dr. Tindall: Please not in the case of the Zinc preparation It has been shown that the Zinc preparation, if it is actually frozen, that is to say if it reaches 0, there is a very rapid deterioration. On the other hand at room temperature the preparation will remain constant for 2 or 3 years.

Dr. Ross:—and the lyophilized powder?

Dr. Stack-Dunne: That is almost indefinitely stable

Dr. West: What do you do then, do you actually incubate it, to get your accelerated test?

Dr. Stack-Dunne: At 37° centigrade we have done some tests That is a temperature below denaturation We also have tests now at room temperature

Dr. West: At 37° do you find that it goes off in months? or weeks?

Dr. Stack-Dunne: It goes off much quicker, appreciably in one month

Dr. Dixon: Is there any knowledge of what this "going off" is? because with pure corticotropin you can get 70 % recovery after 80° centrigrade for a day, under rather precisely defined conditions

Dr. Stack-Dunne: No We do not know what happens

Dr. Tindall: We have had one batch that appeared to have gone off and it was shown to have increased its proportion of A_2 to A_1 from the original one quite materially It looked as though there was a shift in the direction A_1 towards A_4

Professor Symington: Thank you very much Dr Stack-Dunne. We will adjourn for coffee

X-ray *effects that* cell damage results in the release, possibly, of some oxidizing substance If this could be correlated with your idea of oxidizing ACTH, in some way or other, it might account for the loss of effectiveness in the Saffran Schally assay. We have not got very far with this but there is some evidence that damaged tissue, damaged cells in particular, can exert a very local influence, of a degenerative nature and this could well be an oxidative one.

Dr. Grant: That is to say that it tends to inhibit corticosteroid activity.

Dr. Hemingway: *It could do, that is what I have been finding There is some* more evidence recently of Fisher & Fisher who have published a paper in Cancer Research Merely handling liver after injection of latent tumor cells, or tumor cells that are inactive, these cells which are presumably restrained by normal inhibitions, (which we have been taking as partly steroid influences) have ceased to be inhibited and have then started to grow It has not been worked out yet but it is quite interesting evidence

Dr. Grant: You remember the extraordinary thing when Pincus & Hechter, ten years ago, were starting their adrenal perfusion experiments They perfused the wrong way round, as they had to from the anatomy, they perfused into the vein and then because they could not get the perfusate out they made razor slashes into the gland Pincus came to give us a lecture in 1952 He said that *if they took these ox adrenals and put them on a board and beat them like* steaks they actually worked better

Dr. West: Do we infer from what Dr Grant said that using scissors is less traumatizing than using a very sharp razor blade I would have thought that it would have been the other way round

Dr. Grant: I think, Dr West, if you use a really sharp razor blade you are all right I think that Dr Hutchinson in Glasgow was using rather a dull scalpel But Dr Murray Saffran certainly had some very sharp thin bladed scissors, like iridectomy scissors which nipped the adrenals in two I think that if you use a really tharp new razor blade you will be all right.

Dr. Stack-Dunne: I think that it is so, if you snip you get a clean cut right across but if you cut, even with a very sharp blade, you are apt to squeeze and roll If you snip, it starts at one edge and goes right through It looks much cleaner after a snip than after the best cut that you can do

Dr. Dixon: The damaged adrenals gave more or less corticosterone?

Dr. Grant: The funny thing is that in the Saffran assay the damaged adrenal, cut with a blunt scalpel, gave less corticosteroid This has got no relation whatever *to the* other story of Pincus in the perfused gland where when they knocked them about they got a better output

Dr. Dixon: Because the Pincus story fits in with the fact that in the adrenal homogenate there is a very high corticosteroid production, compared with preparations with intact cells, and is no longer increased by corticotropin The corticotropin is bringing the intact cell towards the unleashed production of the homogenate

Mr. Forrest: How do you know that in fact Saffran was not traumatizing his adrenals with his scissors?

Dr. Stowers: Is there any evidence that the anti-body formation is less pronounced with the more active preparations of ACTH?

Dr. Stack-Dunne· I hope that that is going to be discussed this afternoon I don't know at the moment

Dr. West: I would like to ask one more question of Dr Stack-Dunne. You mentioned that we should keep a gelatin preparation, and most of the preparations we have are gelatin, in a *refrigerator* We have not done this with the ordinary

THE USE OF URINARY STEROID ASSAYS TO DETERMINE THE EFFECT OF CORTICOTROPIN IN CLINICAL PRACTICE

H. F. WEST

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Ten years ago Dr. Max Reiss gave me some of his crude corticotropin to give to a patient who had ankylosing spondylitis Ever since then, with a short break in 1950, I have used corticotropin and used a great deal, so I have been naturally very concerned with the methods used to control the therapy. In 1951 we only had 17KS assays and eosinophil counts, neither of which were very helpful In 1952 Dr. Norymberski provided us with the 17KGS (Norymberski et al. 1953) assay and in 1953 with the total 17(OH)CS assay (Appleby et al. 1955). We used these assays as a measure of cortisol secretion. The validity of the assumption may be considered as follows

1. Are the 17KS measured derived from cortisol? The available evidence, and there is a great deal, shows that in the main they *are*, except in rare adrenocortical dysfunctions The colour reaction used is fortunately one of the most specific but two possible interfering chromogens have recently been noted Dr. James told me last night that *old* yeast, that may be used to get rid of glucose from the urine, may develop a Zimmermann chromogen. The other chromogen, according to a recent report, can come from oleandomycin.
2. To arrive at an accurate quantitative figure the chromogenicities of the 17 KS measured should be all the same. It is known that 17 KS with an 11-hydroxyl function are less chromogenic in the Zimmermann reaction than those with an 11-keto function. The strong acid hydrolysis is known to produce an artefact—an 11-dehydro-delta 10–11 product—which is more chromogenic than the 11-keto derivatives Dr. Bush has suggested that at high levels of secretion relatively more 11(OH)17KS than 11-keto may be formed, also that the configuration of the 11(OH) may affect the yield of the dehydro artefacts. I doubt on clinical grounds, whether these chromogenic differences have misled us in our use of the assay for the control of corticotropin therapy

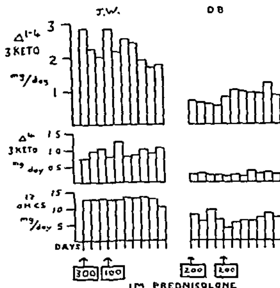


Fig 1.

The 24 hour urinary excretion of unconjugated Δ4 4–1, 3-ketosteroids and Δ4, 3-ketosteroids, and the excretion of total 17-hydroxycorticosteroids following intramuscular injections of prednisolone acetate in two patients

In regard to the quantitative recoveries of metabolites of administered steroids we have encountered recently an interesting and unexplained phenomenon. We have been measuring the urinary excretion of unconjugated steroids with a delta 1–4, 3-keto configuration, to assess the rate of absorbtion of prednisolone trimethylacetate from intramuscular depots. The method is based on an isonicotinic acid hydrazide reaction You will see from the slide (Fig 1) that both patients have had 400 mg. of trimethylacetate in the space of a few days and that one excretes three times as much delta 1–4, 3-keto steroid as the other. We do not think that interfering chromogens are concerned. The solvent + extract blank estimation is high when oranges have been eaten, as is the delta 4, 3-keto steroid INH chromogen. Both these patients had steady low solvent/extract blank readings It might be thought that one absorbed the steroid faster than the other but this is not so for they have been treated continuously with three weekly injections for 4 months and their 'outputs' have remained constant.

In determining the effect of corticotropin therapy the 'output' of corti-

3. Having considered these points the first thing that we had to find out was whether the endogenous and exogenous cortisol was converted to 17(OH)CS in a constant proportion. We gave cortisol by slow intravenous infusion, mimicking the adrenal secretion of cortisol and obtained 40 mg of 17(OH)CS from 95 mg. of cortisol given (All our 'recoveries' were a little higher when the method was modified). We also gave cortisone aldehyde intravenously and cortisone acetate and cortisol by mouth All the 'recoveries' were approximately the same. We also gave cortisone acetate in gradually increasing and decreasing doses If the figures for dose and 17KGS excretion are plotted against each other we find a slow rise in the curve of 17KGS which becomes a straight line when the dose rises above 62½ mg. *This straight line when projected backwards passes through the origin.* We have done many 17(OH)CS urinary assays on patients taking cortisone acetate or cortisol since. They have all fitted onto this curve fairly well. Nevertheless, doubts have arisen about this assumption Gemzell in 1956 described the effect of intramuscular testosterone on plasma levels of Porter-Silber chromogens and on urinary 17KGS recoveries following 100 mg of cortisone acetate (by mouth). We were unable to confirm a major fall of urinary 17(OH)CS and Migeon found the fall in plasma 17(OH)CS a very transitory thing Recently Mills (1959) noted the 17(OH)CS 'recoveries' on a patient receiving cortisone acetate and concurrent oestrogen therapy. He found an early fall in the 'recovery' which gradually returned to normal but which rose temporarily to about 70 % when the oestrogen was withdrawn after some six months therapy He was using a relatively large dose of oestrogen The doses commonly used, except in the treatment of some cancers, do not appear to affect the 'recovery' materially. My own doubts arose when I was testing the absorption of enteric coated capsules of cortisol three years ago. My 'recovery' of 17(OH)CS was 55 % whether I swallowed the capsules whole, chewed them up or took tablets (100 mg. dose) A patient used as a control excreted 45 % as I had done in previous years This finding remains unexplained—it has been checked again recently Dr Peter Davis, who is here, tells me that he has experience of a number of rheumatoid arthritic patients who return, as 17(OH)CS, only 20–30 % of a dose of 100 mg. of cortisone acetate. I have not met this sort of thing. *If there is no other explanation* (for his figures) than an altered metabolism of cortisone it is a very important finding

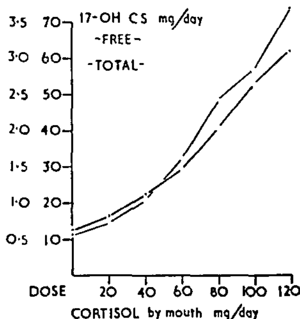


Fig 3
To show the relative increase in unconjugated (free) and 'total' 17-hydroxycorticosteroids See fig 2

taken in divided doses by mouth for two days at each dose level—all days were consecutive. You will observe the rate of increase of 17(OH)CS metabolites and the rate of increase of cortisol excretion I showed this figure to Sandberg, who has studied protein binding more than most, I think. He said that the cortisol curve fitted his findings for the protein binding of cortisol This suggests that the excretion of free cortisol is a much more sensitive measure of the cortisol available to the tissues than the excretion of 17(OH)CS. With regard to the absolute figures for cortisol excretion Schedl and co-workers (1959) have found that approx. 80 % is reabsorbed by the renal tubules at physiological levels This figure is compatible with our findings. They also reported, on limited data, that neither glomerular filtration rate nor urine volume had much effect upon the amount excreted. (In view of the discussion that followed this paper I have included figure 3 to show the excretion of free 17(OH)CS in the same experiment).

The next slide (Fig. 4) shows the relation between cortisol plus cortisone excretion and the excretion of total 17(OH)CS in patients treated

sol may not always be the right parameter to measure. In spite of the value of the urinary 17(OH)CS assay in control of therapy we cannot use it to *prescribe* therapy. We cannot say that you should give such a dose of corticotropin as will produce 25 mg. of 17(OH)CS in the urine. I suspect that this is not *solely* because disease states vary in severity and one patient needs a higher level than another. I have favoured the idea, for some years, that steroids operate as catalysts—I would like to hear this subject discussed—and that it is their concentration in the tissues that matters, not their turnover or daily production rate. Recent studies have shown that cortisol is protein bound in the plasma and that its effectiveness is related to the concentration that is not so bound. Slaunwhite & Sandberg (1959) have named the binding protein transcortin—because it is virtually specific for cortisol. For more than three years now I have been of the opinion, based on the work of Venning and others, that the free cortisol and cortisone to be found in urine may reflect the concentration of unbound diffusible cortisol in the blood. The next slide (Fig 2) shows the results of an experiment designed to test this idea. Cortisol was

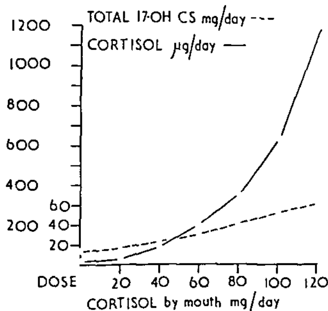


Fig 2

The 24 hour urinary excretion of cortisol and of total 17-hydroxycorticosteroids in a normal individual taking corticol in divided doses by mouth Each point is the mean of two consecutive days at each dose level

ceeding the dose of corticotropin that corresponds to the point of the curve where the free cortisol excretion starts to rise rapidly.

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DISCUSSION FOLLOWING PAPER BY DR. WEST

Dr. Davis: I have got the values that we found for the 17(OH)CS excretion in patients on oral cortisone acetate or hydrocortisone (See Fig 1) This was a random group Some of them had been on treatment for about a week and some for up to 6 years They are not just isolated readings, they are all at least averages of 3 or 4 over a week or two, and some over several months As you see many of them are falling below the expected level of 45 % 'recovery' * which should give a figure of 22 mg for 75 mg or cortisone acetate Quite a lot of them are well below that mark—down into the thirties or twenty percent 'recoveries' What is interesting is that when I came to try to relate this finding to the incidence of side-effects, they seemed to be related to the 17(OH)CS excretion rather than to the dose that was being given As you see the black dots are for those people with definite side-effects, I don't mean severe ones, side-effects such as you could say by looking at them that they are probably having cortisone The slight degree, patients whom you had known before treatment and could therefore detect a slight degree of "mooning". The X's had none at all. As you see, with an excretion above 20 mg 17(OH)CS a day side effects begin to appear We would agree with Dr West, that if you get above 30 mg a day consistently, they certainly have side-effects We have used the 17(OH)CS assay for the last 5 years in a very similar way to Dr West and we have found it invaluable clinically despite this possible discrepancy We have used it mainly, I think, to try to guard against inducing side-effects in our corticotropin patients We have not been at all worried if their clinical course has been satisfactory with a low corticosteroid excretion We do know that if their excretion starts climbing above 30 mg we have got to do something about it This might explain it In some way it is the changes in metabolism that affect the 17(OH)CS excretion which we need to be worried about in practise, and that may, as you say, be in some way related to the free cortisol

Could I put up another [illegible]

* Theoretical recovery of cortisol in the *form* of 17(OH)CS, not recovery of x mg. of 17(OH)CS as used by Dr West, i e 34 mg

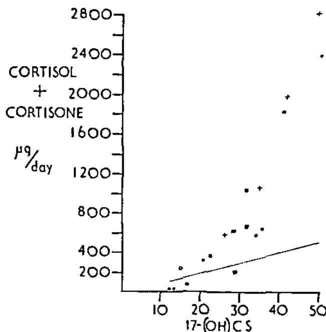


Fig 4

Some random assays for urinary cortisol and cortisone in patients on corticotropin therapy plotted against their excretion of total 17-hydroxy corticosteroids

with corticotropin We have few results as yet and you will see that some of them do not fit the expected curve very well We do not know why this is yet but one patient was of particular interest. She had severe widespread aggressively active rheumatoid arthritis and had been running a temperature for months. Her daily excretion of 17(OH)CS was 13–14 mg On corticotropin therapy she made the usual dramatic recovery and eventually showed signs of overstimulation but her 17(OH)CS excretion was only 16 to 19 mg. These figures, in our experience, showed too small a rise to account for such a major change in the clinical picture The figure (4) shows that her excretion of cortisol (open circle) was at a level that in others would have been associated with a 17(OH)CS excretion of at least 25 mg

In conclusion I would like to say three things (1) The 17(OH)CS assay is a very useful guide for corticotropin therapy. (2) More work is necessary to sort out the percentage 'recovery' as 17(OH)CS, of administered and endogenous cortisol. (3) That in long-term therapy beware of ex-

ceeding the dose of corticotropin that corresponds to the point of the curve where the free cortisol excretion starts to rise rapidly.

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DISCUSSION FOLLOWING PAPER BY DR. WEST

Dr. Davis: I have got the values that we found for the 17(OH)CS excretion in patients on oral cortisone acetate or hydrocortisone (See Fig 1) This was a random group Some of them had been on treatment for about a week and some for up to 6 years They are not just isolated readings, they are all at least averages of 3 or 4 over a week or two, and some over several months As you see many of them are falling below the expected level of 45 % 'recovery'* which should give a figure of 22 mg for 75 mg or cortisone acetate Quite a lot of them are well below that mark—down into the thirties or twenty percent 'recoveries' What is interesting is that when I came to try to relate this finding to the incidence of side-effects, they seemed to be related to the 17(OH)CS excretion rather than to the dose that was being given As you see the black dots are for those people with definite side-effects, I don't mean severe ones, side-effects such as you could say by looking at them that they are probably having cortisone The slight degree, patients whom you had known before treatment and could therefore detect a slight degree of "mooning". The X's had none at all As you see, with an excretion above 20 mg 17(OH)CS a day side effects begin to appear We would agree with Dr West, that if you get above 30 mg a day consistently, they certainly have side-effects We have used the 17(OH)CS assay for the last 5 years in a very similar way to Dr West and we have found it invaluable clinically despite this possible discrepancy We have used it mainly, I think, to try to guard against inducing side-effects in our corticotropin patients We have not been at all worried if their clinical course has been satisfactory with a low corticosteroid excretion We do know that if their excretion starts climbing above 30 mg we have got to do something about it This might explain it. In some way it is the changes in metabolism that affect the 17(OH)CS excretion which we need to be worried about in practise, and that may, as you say, be in some way related to the free cortisol

Could I put up another slide, changing the subject slightly It may provide some evidence for another point you made The question of clinical response and 17(OH)CS excretion As I say we have not made any attempt to keep the corticosteroid excretion up to any given level in our rheumatoid arthritic patients If it has been low and they

* Theoretical recovery of cortisol in the *form* of 17(OH)CS not recovery of x mg. of 17(OH)CS as used by Dr West i.e. 34 mg.

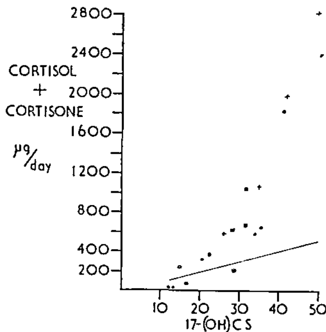


Fig 4
Some random assays for urinary cortisol and cortisone in patients on corticotropin therapy plotted against their excretion of total 17-hydroxy corticosteroids.

with corticotropin. We have few results as yet and you will see that some of them do not fit the expected curve very well. We do not know why this is yet but one patient was of particular interest. She had severe widespread aggressively active rheumatoid arthritis and had been running a temperature for months Her daily excretion of 17(OH)CS was 13–14 mg On corticotropin therapy she made the usual dramatic recovery and eventually showed signs of overstimulation but her 17(OH)CS excretion was only 16 to 19 mg These figures, in our experience, showed too small a rise to account for such a major change in the clinical picture The figure (4) shows that her excretion of cortisol (open circle) was at a level that in others would have been associated with a 17(OH)CS excretion of at least 25 mg.

In conclusion I would like to say three things (1) The 17(OH)CS assay is a very useful guide for corticotropin therapy (2) More work is necessary to sort out the percentage 'recovery' as 17(OH)CS, of administered and endogenous cortisol. (3) That in long-term therapy beware of ex-

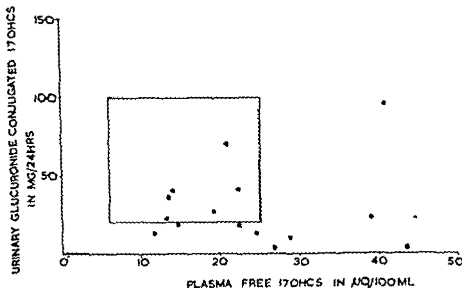


Fig 2

Assays from patients with wasting from various chronic diseases Ordinate —urinary Porter-Silber chromogen after glucoronidase hydrolysis Abscissa —Plasma free Porter-Silber chromogen The box covers the average normal findings

the impression that the people who were doing this sort of thing were the people with severe disease who were responding fairly well to corticosteroid

Dr. Shuster: We have some evidence that may be relevant to the reduced recoveries in some of these patients We have been studying patients with wasting from various chronic diseases, where the basal urine output of corticosteroid metabolites is low—measured by various ways—Norymberski and Porter-Silber These patients have in fact a plasma concentration of free 17(OH)CS (Porter-Silber) which is normal or high So that it looks as if these patients have an abnormality of metabolism of the hormone Now these people look as if they may have Addison's disease, many of them There is certainly never any evidence of the opposite—never any evidence of Cushing's syndrome despite this high plasma concentration of the free 17(OH)CS Associated with this the free Porter-Silber steroids in urine are normal or increased This is very similar to the situation in pregnancy where the urinary output of conjugated steroids may be normal but the plasma free steroid is usually increased In these patients it has recently been postulated that there is an increased protein binding of cortisol So I think that in many of these patients with chronic wasting disease there may be an increased cortisol binding and a reduced output of conjugated steroids in the urine which could give you an excretion of less than 45 % of administered cortisone I think that it is most likely that many of your chronic rheumatoids have a similar abnormality of cortisol metabolism perhaps either hepatic or due to protein binding Here I have to disagree with Dr West because there is a normal or increased output of the free steroids, in wasted patients, as if these protein bound steroids are still excreted normally by the kidney The point being that I do not

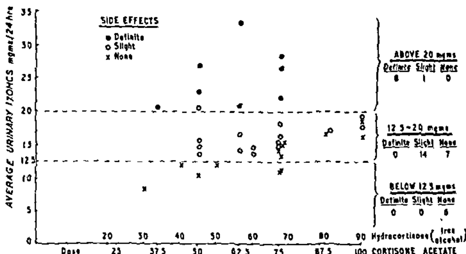


Fig 1

To show the relation of side-effects to the excretion of total 17-hydroxy corticosteroids and to the dose of cortisone acetate or hydrocortisone given by mouth

have been responding quite satisfactorily we have been happy. We have had a number of people who seem to respond in 17(OH)CS within the normal range and I think that this rather illustrates the point (see fig 1 in paper by Dr. Davis) This patient who was stimulated well to begin with responded well, as you would expect. The upper part of the chart shows the response of grip, tenderness and E.S R After a month or two the 17(OH)CS excretion fell off down into the normal range so ACTH was stopped, but as you see there was a very sharp clinical relapse The E S R shot up to 70, the grip came down and the 17(OH)CS excretion fell right away She was put back on quite a small dose, responded and returned to her previous state I think that there is no doubt that these 17(OH)CS figures round about 10 do mean that the ACTH was doing something Again possibly it is a question of the free cortisol question Having pointed out these discrepancies I must emphasize again that the test has been invaluable in practice

Dr. West: Could we have the first slide again? I thought of this slide that one need not bother about the apparently high recoveries because it is possible that their adrenals were contributing Of the low 'recoveries' I have not observed this sort of thing in my patients

Dr. Davis: Yes, I think, certainly, that we can ignore those people because their adrenals may be contributing But most of them fell below—particularly those people over here (to the right below the 45 % recovery line) who are on 100 mg of cortisone acetate. They are people with severe disease activity I think two of them were cases of severe collagen disease, rather than just ordinary rheumatoid arthritis, otherwise we would not be giving them that dose persistently They were well below the line This question of whether you can relate this to the severity of the disease is a difficult one, it is difficult enough to measure the clinical state of the patients in these diseases To take off the veneer of the suppression that you are producing with cortisone and to estimate the underlying severity of the disease is, I think, virtually impossible But one does get

primarily concerned with here. Now he has suggested and shown very well that there is a recovery around 45 % of injected cortisol at high levels of cortisol, but he has raised doubts as to whether this applies in all cases and all down the line Dr Davis has suggested that clinical responses have been obtained with no rise in output of total 17(OH)CS or 17KGS—at any rate sometimes.

Dr. Davis: I would not say definitely no rise but below the level normally obtained

Dr. Cope: Now what seems to be happening is that people are trying to read into the data speculative phenomena, to a certain extent, below the normal levels of adrenal activity. Above that, when you give large doses of cortisone, you can get a recovery of 50 % by this method But when you get below an output of 40 mg or so, you don't necessarily get that recovery and you speculate. You say this is because the patient's endogenous adrenal is producing something, a theory that is rather convenient because you have no evidence either way and you can speculate as you like

Dr. Davis: We were arguing, sir, on my slide, on nothing below 75 mg.

Dr. Cope: Yes I know The point I am trying to make is that there are no adequate parameters involved in this and there are these days much more rigid and valuable methods, using the isotopic methods, which give you a far better arbiter of these other tests. One does not suggest that they are absolutely inviolate but they go a very long way towards specificity, far further than these global methods do Now we have compared the 17-KGS outputs with isotopically

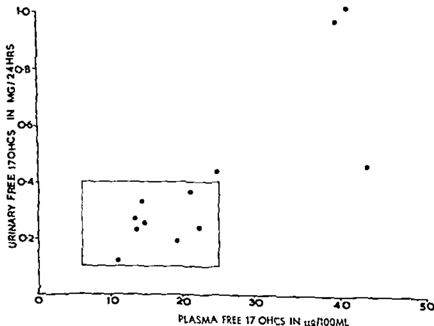


Fig 3.

Ordinate —Free (unconjugated) urinary Porter-Silber chromogen, otherwise as in Fig 2.

think that the urine output of free unconjugated steroid necessarily reflects the protein binding

Dr. West: May I get it right—you said that some of these patients with chronic debility—their blood level was high and their urinary output was normal both of conjugated and free?

Dr. Shuster: No I have a slide if it will make it any simpler. (See Fig. 2) The box shows the average normal range You see that about half of the patients have a below normal output of conjugated Porter-Silber chromogens and a fair proportion of them have plasma concentrations above normal. At the same time the total 17(OH)CS, Norymberski, are equally low In contrast to this the plasma cortisol is normal or increased When you come to the free urinary steroids (Fig 3) you see that the increase in the urine is in proportion to the increase in the plasma So that this is a situation again that occurs in pregnancy, where you have an increased output of the free steroid but a normal conjugated—and the plasma is away up I think that it is likely that we have the same sort of thing in chronic wasting diseases —an increase in protein binding of cortisol with a reduction in the rate of metabolism in the liver The important point is that I think we cannot assume that urinary output of free unconjugated steroid reflects the protein binding There is no good data so far on how cortisol is excreted by the kidney It could be, it is possible, that it is secreted by the tubules The fact that protein bound cortisol is apparently so easily excreted by the kidney (but not metabolized by the liver and is presumably not filterable) is very suggestive that the kidney is capable of secreting it

Dr. Stowers?: What is the thyroid function like in these people?

Dr. Shuster: We have not measured it It has been measured by other people, and tends to be below normal

Dr. Stowers?: If the thyroid function is below normal this would tend to decrease the turnover rate of your cortisol

Dr. Shuster: It was nothing of that order

Dr. West: You say "free 17(OH)CS". What is that?

Dr. Shuster: Porter-Silber chromogen

Dr. West: That is entirely different from the excretion of cortisol

Dr. Shuster: Yes, we have done a few measurements of cortisol and cortisone—chromatographically, while I was in Dr Cope's laboratory—they worked out roughly the same

Dr. West: We find that the output of free delta 4, 3-keto steroids, also the output of free 17(OH)CS (Norymberski)—the *rate* of *increase* is the same as that of the conjugates

Dr. Shuster: The rate of increase with increasing dose in a particular patient? That is a rather different situation These are different patients with various wasting diseases It is in these patients that these two do not correlate In an individual patient I expect that you will get a straight line These are the same patients as in the previous slide and they have a high urinary output—or normal—of free steroid but low of conjugated I think that this is a fairly universal pattern

Dr. West: These free steroids you have got here What are they? You have got reduction at 20, (not in P-S reaction) you have got free unconjugated tetrahydro F and tetrahydro E—they are a whole collection

Dr. Shuster: Yes. This does work too with directly measured cortisone and cortisol

Dr. Cope: One of the main themes of this talk is the reliability of methods, in clinical and in any deductive function I think that is what Dr West is

CORTICOTROPHIN AND ALDOSTERONE SECRETION

BERTHA SINGER
Dept of Medicine University of Sheffield

I must thank Dr. West for inviting me to this conference to hear about the latest work on corticotropin. As I have been spending my time on the physiology of aldosterone I have very little personal experience with ACTH. However, as I'm 'on the spot' as it were, Dr. West just couldn't resist the opportunity of asking me to give a short summary of recent work on ACTH in relation to aldosterone production, a subject, he feels, I should be interested in. Therefore, what I have to say will probably be known to you already, but I hope it will serve as an introduction to a short discussion on the subject.

The main point about aldosterone production is that it appears to be largely independent of ACTH This feature of adrenal function was surmised long before aldosterone itself was discovered and was based on the fact that a hypophysectomised animal did not require an excess of salt in the diet in order to survive, whereas an adrenalectomised one did. This suggestion was supported later by the work of Deane, Greep and others who demonstrated, in the rat, by histological and histochemical techniques, that the zona glomerulosa did not atrophy after hypophysectomy and from this deduced that this zone must be producing a salt-retaining hormone. This work was of course confirmed much later by Giroud et al. and Ayres and the Taits who showed, in-vitro, in the rat and in beef adrenals that aldosterone production is primarily confined to the zona glomerulosa

A further indication of the relative independence of aldosterone with respect to ACTH is the fact that hypophysectomy resulted in a much lesser fall in aldosterone secretion into the adrenal vein blood than that of other corticoids This was shown in the rat by Stack-Dunne and myself, and by Farrell and his group in the dog In the dog the fall after hypophysectomy and the response to ACTH were not quite as great as in the rat.

In man there is considerable evidence that a subject with pituitary insufficiency can adapt himself to decreased salt intake. This adaption can be shown to be associated with an increase in urinary aldosterone

measured cortisol production and we get, as other people do, around 50 %, with large turnovers, about 50 % comes out as 17-KGS But with lower turnovers, below 40–50 mg production a day, or oral dosage a day, the relation between the two gets progressively worse, at least in our hands—which you can call an average laboratory technical skill for 17-KGS. At the ordinary resting levels of adrenal activity, very little relation at all exists between the apparent 17-KGS and the measured cortisol production All I am suggesting now is that there are far better parameters and a lot of these speculative points can be resolved The methods are not difficult and they will bring the thing a long way forward They are applicable in the presence of all the other steroids. You can measure quite easily your suppressed adrenal in the presence of prednisolone, you can tell how much the adrenal is producing and how much the prednisone is producing. Dr. West gave some figures on the effects of prednisolone trimethylacetate Now it is quite easy to measure prednisolone and prednisone excretion in the urine It is a much more specific measure of what is happening under those circumstances, at least as regards the metabolism of prednisone I suggest that these things ought to be done much more, rather than speculate as to what is happening to a very complex thing like the 17KS total assay, which has many other variables involved in it at the lower levels

Dr. Davis: At the upper levels that you were talking about, sir, were these your figures, of a fairly constant 50 %; did that relate to seriously ill patients as well?

Dr. Cope: Well they were ill It does not seem to make a lot of difference

Prof. Symington: Ladies and gentlemen I am sorry to cut this discussion short We have still another paper There will be time to carry on this discussion after 5 o'clock—under the general discussion. I think that we will have an ACTH committee and it looks as though we will have to have another committee of Dr Cope and Dr Davis to report back here at 5 o'clock We have got Dr. Norymberski here—would you like to make any comment before we call on Dr Singer

Dr. Norymberski: Yes, on what the method really measures With the qualification that in most circumstances it does measure the secretion of cortisol, I think that it is dangerous to assume this in relation to corticotropin therapy, because of the type of steroid formed on adrenal stimulation at the threshold of the various enzymes involved in the biosynthesis of cortisol I think that there is quite a bit of evidence in the literature that on treatment with corticotropin you do get very large amounts of other secretory products, other than cortisol, which might be partly responsible for sideeffects What the method really does measure, and that we feel certain about, in the absence of extra adrenal activity, is the activity of the 17-hydroxylase I think that is important to remember, particularly in pathological states

Dr. Fotherby: Could I add one word sir? We have been interested in estimating pregnanetriol We find that in normal people the amount of pregnanetriol excreted is about 10 % of the total 17(OH)CS In addition to the pregnanetriol there is the 17-hydroxy pregnanolone and various other 17 hydroxy steroids that contribute to the total 17(OH)CS estimations So that perhaps not more than 80 % of the total 17(OH)CS that you measure in urine do in fact come from cortisol The other 20 % comes from other steroids secreted by the adrenal

Prof. Symington: Reluctantly I have to close this paper Thank you very much Dr. West I will call on Dr Singer to speak on corticotropin and aldosterone secretion

I feel a few words should be said about the difficulty of interpreting results based on urinary excretion, secretion into the adrenal vein blood by the surgically stressed animal, or by *in-vitro* techniques All of these are open to serious criticism and could give misleading results. For example, ACTH has no effect on aldosterone production by *in-vitro* techniques, it has an effect in surgically stressed hypophysectomised animals when adrenal vein blood is studied, but not in intact animals under these experimental conditions. In man an increase in aldosterone can be demonstrated in the urine of normal subjects treated with ACTH but it appears to be a small effect and short-lived Thus each method has given a somewhat different result and one wonders which of these is closest to what happens under physiological conditions There is little information on this point, although some recent work from Australia is of great interest. MacDonald and Reich have reported on aldosterone secretion in the conscious unstressed sheep in which the adrenal was transplanted to a carotid-jugular skin loop. They were unable to detect any aldosterone in the unstressed animal Sodium deficiency resulted in the appearance of aldosterone in the adrenal vein blood. ACTH had no effect on the secretion of aldosterone unless the animals were sodium-deficient. Unfortunately, the authors have not studied the animals following surgical trauma, so it is difficult to transpose this information to the dog or rat It is possible that aldosterone would not be detectable even in the surgically stressed sheep, in which case this animal would differ from the dog and rat. However, this work sounds most promising, and it may help to clarify some of the conflicting results obtained by other techniques

I should like to end by making a suggestion, which you will no doubt demolish, that aldosterone secretion is probably entirely independent of ACTH I suggest that the decrease in aldosterone secretion in the hypophysectomised surgically stressed animal is probably related to something quite different, such as reduced blood flow and that the response to ACTH administration is due either to an effect on blood flow or to contamination with 'glomerulotrophin'

On the point about ACTH and adrenal blood flow I should like to point out that I have examined our own published results on the adrenal blood flow and although it is possible to alter aldosterone secretion by the administration of DOCA or with synthetic diets, without altering the adrenal blood flow, there is a definite difference in the blood flow of normal rats, hypophysectomised rats, and hypophysectomised rats treated with ACTH.

A point reported by Muller, which is of some interest, is that some subjects with pituitary insufficiency had no diurnal rhythm until prednisolone was given—suggesting that ACTH may play a part in the diurnal variation in aldosterone production. ACTH administration in normal subjects does appear to increase urinary aldosterone excretion. The extent of the increase and the length of time which it will act has varied considerably in different studies Generally, the effect is more short-lived and of lesser degree than the response of the other corticoids. The response appears to be greater when subjects are on low salt intake than on normal diet.

How ACTH exerts this somewhat limited effect on the adrenal is at present unknown. Giroud and his group were unable to show an increase in the *in-vitro* production of aldosterone by rat or beef adrenal slices by addition of ACTH to the incubation medium, although it did result in an increase in the production of other adrenal steroids. If ACTH acts very early on in the synthesis of the corticoids, such as the breakdown of the cholesterol side chain, it is difficult to see why it doesn't affect aldosterone production One possible explanation would be that the zona glomerulosa does not respond to ACTH No doubt Dr. Grant and others will have some ideas on this point

I have been referring to ACTH in general but I should mention that not all preparations have the same relative effect on aldosterone and hydrocortisone or corticosterone Bell, in the United States, has prepared two types of ACTH one of which is referred to as beta and the other as delta$_1$ Farrell has studied the effect of both preparations on the adrenal secretion of aldosterone and cortisol in the decerebrate dog Delta$_1$ corticotropin was several times more potent than beta corticotropin with respect to aldosterone stimulation However, it does not appear that this substance is a special 'aldosterone stimulator', firstly because it also has a potent effect on cortisol secretion, and secondly Farrell has shown that extracts of the diencephalon contain a substance which is considerably more potent than delta$_1$ ACTH in stimulating aldosterone secretion This substance appears to be concentrated in the pineal gland and surrounding tissue, a point which is of great interest to biologists in general. As the nature of this substance is not known it is not possible to say whether there is overlapping of activity between it and ACTH, as with oxytocin and vasopressin, or whether delta$_1$ corticotropin is actually contaminated with 'glomerulotrophin' as Farrell has named his active substance from diencephalic extracts. This latter hypothesis would appear to be a much neater one and is possibly more attractive.

but less than that on the fasciculata. I don't know whether you should say that is has *no influence on it. I think that it probably has some influence on it, and very likely it* has some effect on the secretion of aldosterone in the normal animal.

Dr. Tait: I have very little positive to add to this excellent review by Dr. Singer. In reviewing the literature it is a very confusing subject. Any positive result can be explained in terms of contamination of the ACTH. This will be only an academic point; we don't know whether the contamination is always secreted with the ACTH Any negative result can be explained, as Muller and Bartter and Liddle have done by a negative feed-back system The increase in aldosterone or in corticosterone, automatically reduces the output of aldosterone. I think that there is now general agreement that (a) ACTH has very little effect on aldosterone secretion by the gland in-vitro and (b) that it can have quite a considerable effect in a human on a low salt diet. This can be a fivefold increase or something of that kind. For some years, with Dr. Hechter's group, we have been trying to explain this lack of ACTH effect, if it is a lack, in terms of a biosynthetic scheme. We have really failed in this From our knowledge of the scheme there is no reason why ACTH should not have an effect on aldosterone secretion In other words if one accepts, and we shall have to wait for Dr. Grant's paper before fully doing this, that ACTH works between cholesterol and progesterone I think that there is now general agreement between our group and Dr Giroud's that cholesterol *is* a substrate for aldosterone biosynthesis in the glomerulosa Therefore we have to come to some unsatisfactory explanation that ACTH does not attach itself to the glomerulosa or not so much as to the fasciculata To my knowledge no secretion rate studies have been done on this low salt diet with the effects of ACTH, but this fivefold increase looks to be quite convincing It does seem that there is some synergistic action between say glomerulotropin and ACTH I don't think that this need be so mystical in terms of a biosynthetic scheme. For instance one could say that the rate limiting step ordinarily in the biosynthesis of aldosterone is between corticosterone and aldosterone Perhaps the enzymes are saturated or partially saturated and ACTH has not got very much effect at normal secretion rates On the other hand if this step from corticosterone to aldosterone is boosted by a low salt diet then the supply of corticosterone may become rate limiting and therefore ACTH would have a greater effect. This is all speculation but at least it does show how we could explain the present results The snag to that type of explanation is that, at least in-vitro, although ACTH doesn't have en effect, we know that the enzyme stimulates the production of corticosterone in quite reasonable amounts, if you can tell that the enzyme is not saturated However I do think that there is beginning to be a lot of evidence that the adrenal gland in-vitro is quite abnormal in a number of ways. Dr. Hechter has recently found that the permeability of the gland, as one might expect, is entirely different in-vitro compared with in-vivo That is all I have got to add.

Prof. Symington: I would like to ask the meeting in general—are we to accept glomerulotropin, is it quite respectable?

Dr. Tait: I don't think that there is any evidence whatsoever that this is the compound that increases on a low salt diet, nor do I think that Dr Farrell would suggest this. It is just something which he gets with quite reproducible results, that is all

Dr. Grant: "A factor"

Dr. Cope: Could I ask Dr. Singer one point that I may have missed. If ACTH, in her experiments, is producing any stimulation of aldosterone under those rat circumstances, you ought to be able to show a change in aldosterone pro-

5	expt.'s normal rats	16.5 ml/adr./kg /hr.
9	– hypox (2–44 days)	9.4 –
5	– – + acute ACTH	2 days after op. (2) 12.2 mls
		7 – – – (3) 10.1 –
4	– – + ACTH 7 or 14 days	15.4 mls.

Recently Sapirstein and Goldman have reported that there is an increase in adrenal flow following ACTH in the rat by the method of uptake of Rb. 86.

DISCUSSION FOLLOWING DR BERTHA SINGER'S PAPER

Dr. Dixon: Two small points I don't think that it is quite fair to call the delta 1 ACTH 'highly purified' Bell's alpha beta and gamma fractions were highly purified, his delta was just what did not move in a counter current, and then on another counter current system he just got a rough separation, quite a few transfers, to separate the delta 1 & 2 So it might easily be contaminated, as you said, by something The other point is that I think W E Balfour with the calf, has got large increases in blood flow with ACTH and in fact increase of the glucocorticoids He finds that in his particular situation the glucocorticoid concentration falls after ACTH, though the output is greatly increased, so big is his increase in blood flow

Dr. Stack-Dunne: Yes, I think that I would like to come in too I forget who it was but someone recently has done similar work on collecting adrenal effluent and they found very little correlation of total output of steroids with blood flow I don't know whether they have tried this on aldosterone as well but it seems that the blood flow is not the limiting factor The second point that I would like to come in on, is this idea that corticotropin has no effect upon the glomerulosa In the rat, at any rate, this is definitely untrue It is a misinterpretation which has been appearing in the literature for many years, based largely on the observation that the width of the glomerulosa stays constant or even increases after hypophysectomy If you calculate the volume of the glomerulosa, considered as a hollow sphere, you find it is in fact reduced and Lever has done very careful morphological studies on the rat showing that after hypophysectomy the individual cell volume in all zones steadily decreases, the cell volume in the glomerulosa decreasing more slowly than in the fasciculata Dr. Cater and I have shown that the rate of mitosis in the glomerulosa falls off markedly after hypophysectomy (and is increased by corticotropin) Some work of Lever showed up rather clearly a vascular block between the glomerulosa and the fasciculata, located in the region of the zona intermedia This block is influenced by corticotropin In hypophysectomised animals the vessels seem to be almost completely occluded in his experiments, whereas in the corticotropin treated animals the vessels became more patent and this went along with the disappearance of the sudanophobe zone I think that it is more likely that corticotropin, assuming—I think that it is well proved in fact—that the glomerulosa produces the aldosterone, that corticotropin has some effect on the glomerulosa,

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9 –	hypox (2–44 days)	9.4 –
5 –	– + acute ACTH	2 days after op. (2) 12.2 mls
		7 – – – (3) 10.1 –
4 –	– + ACTH 7 or 14 days	15.4 mls.

Recently Sapirstein and Goldman have reported that there is an increase in adrenal flow following ACTH in the rat by the method of uptake of Rb. 86.

DISCUSSION FOLLOWING DR BERTHA SINGER'S PAPER

Dr. Dixon: Two small points I don't think that it is quite fair to call the delta 1 ACTH 'highly purified'. Bell's alpha beta and gamma fractions were highly purified, his delta was just what did not move in a counter current, and then on another counter current system he just got a rough separation, quite a few transfers, to separate the delta 1 & 2. So it might easily be contaminated, as you said, by something The other point is that I think W E Balfour with the calf, has got large increases in blood flow with ACTH and in fact increase of the glucocorticoids He finds that in his particular situation the glucocorticoid concentration falls after ACTH, though the output is greatly increased, so big is his increase in blood flow

Dr. Stack-Dunne: Yes, I think that I would like to come in too I forget who it was but someone recently has done similar work on collecting adrenal effluent and they found very little correlation of total output of steroids with blood flow I don't know whether they have tried this on aldosterone as well but it seems that the blood flow is not the limiting factor. The second point that I would like to come in on, is this idea that corticotropin has no effect upon the glomerulosa. In the rat, at any rate, this is definitely untrue It is a misinterpretation which has been appearing in the literature for many years, based largely on the observation that the width of the glomerulosa stays constant or even increases after hypophysectomy If you calculate the volume of the glomerulosa, considered as a hollow sphere, you find it is in fact reduced and Lever has done very careful morphological studies on the rat showing that after hypophysectomy the individual cell volume in all zones steadily decreases, the cell volume in the glomerulosa decreasing more slowly than in the fasciculata. Dr. Cater and I have shown that the rate of mitosis in the glomerulosa falls off markedly after hypophysectomy (and is increased by corticotropin) Some work of Lever showed up rather clearly a vascular block between the glomerulosa and the fasciculata, located in the region of the zona intermedia This block is influenced by corticotropin. In hypophysectomised animals the vessels seem to be almost completely occluded in his experiments, whereas in the corticotropin treated animals the vessels became more patent and this went along with the disappearance of the sudanophobe zone I think that it is more likely that corticotropin, assuming—I think that it is well proved in fact—that the glomerulosa produces the aldosterone, that corticotropin has some effect on the glomerulosa.

Dr. Stack-Dunne: To what extent might that be related to protein synthesis with growth hormone?

Dr. Singer: I think the effect was perhaps greater than one might expect with simple weight change, in the subject as a whole anyway

Dr. Phillips: One of the interesting things about some of my own work is that aldosterone can be produced from an adrenal that has not got a zona glomerulosa. I am speaking of the fish adrenal. I am just wondering whether in these lower vertebrates that have not got zonation in the adrenal glands, whether some of the cells, which look identical with all the others, do in fact produce aldosterone—that there is a qualitative difference of secretion between some cells and others

Prof. Symington: This is an interesting point in view of the discussion we had, Dr Tait, on the appearance of the cell in aldosteronism, because it is not a glomerulosa cell which is apparently forming aldosterone in Conn's syndrome

Dr. Cope: If it is fish surely you have to distinguish between salt water and fresh water.

Dr. Pillips: That is true, the only fish I study is the salmon which is moving from salt water to fresh water

Dr. Grant: It is probably a very active adrenal when they move from salt to fresh water.

Dr. Phillips: Yes In the migrating salmon there is marked hyperplasia.

Dr. Tait: There is a recent report, of the American bullfrog I think, in which aldosterone is the main steroid This has been confirmed

Dr. Phillips: Yes

Prof. Symington: Well ladies and gentemen that concludes the first session Thanks to the speakers for keeping to their time and to you for livening up the discussion

duction by inhibiting the ACTH production with a steroid like corticosterone or one of the modern non-electrolyte ones—like dexamethasone. Have you tried that?

Dr. Singer: We have not tried the modern ones but we did give corticosterone, and were not able to show an effect. Perhaps we did not give enough, perhaps the stress of the operation was so great that it overcame the inhibition. We thought that this might do something, corticosterone being the normal glucocorticoid secreted by the rat

Dr. Cope: It caused no change?

Dr. Singer: We were not able to show any change

Dr. Stack-Dunne: But the stress of the procedure is enormous

Dr. Chalmers?: You attribute the DOCA effect to potassium?

Dr. Singer: Well, sodium retention or potassium depletion

Dr. Ross: If we take patients with long standing hypopituitarism and put them on a low salt diet they will then gradually adapt to their low salt diet and their sodium output may go in company with their intake; but their aldosterone excretion will rise only very slightly. If you take a patient who has been hypophysectomized and do this, – in the one case I have done it was three months after hypophysectomy, – they have a perfectly normal fall of sodium excretion and a perfectly normal rise of aldosterone excretion It looks as though it may perhaps be the size of the gland I don't know how fast that falls after hypophysectomy Something obviously happens slowly because over the years they cease to respond to ACTH

Dr. Singer: Yes, this is what Cater and Stack-Dunne have done. In fact the original work of Deane & Greep is not quite as absolute as one would have thought from their paper The zona glomerulosa does in fact atrophy although perhaps not at the same rate as the other zones

Dr. Cope: We have got to remember that when you do a hypophysectomy under any of these circumstances you do a lot of other things The patient is liable to be myxoedematous for one thing, and myxoedema is going to have quite an effect upon all these tissues we are considering

Dr. Ross: This patient was maintained on thyroid

Dr. Cope: Yes, but there are all the other things as well, growth hormone for instance

Dr. Stack-Dunne: There is, in the experimental animal, the rat, no specific effect of growth hormone on the zona glomerulosa, judged by morphological standards, or on aldosterone secretion

Dr. Cope: Rat growth hormone?

Dr. Stack-Dunne: No—that is a point

Dr. Singer: Well we are using pig ACTH in humans and are hoping to get an effect.

Dr. Cope: Not pig growth hormone though

Dr. Chalmers: Do you attribute the reported effects of human growth hormone on aldosterone secretion to contamination?

Dr. Singer: Well I really cannot say I was in on some of the very earliest work on that with Dr Venning and I must say that I did the assay myself and did find some effect, but at that point the extraction procedures were not as rigorous as they are now I see that Dr Venning has done it much more thoroughly and much more exacting since and she still finds some effect Whether it is from contamination or whether it is an intrinsic effect I would not like say.

Dr. Chalmers: What effect?

Dr. Singer: An increase in the secretion of aldosterone with growth hormone

THE EFFECT OF ACTH ON THE HUMAN ADRENAL CORTEX

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Dept. of Pathology University of Glasgow

The result of stimulation of the human adrenal cortex by ACTH may be studied by indirect or direct methods. Most morphological studies are based on indirect methods and consist of post-mortem examination of the human adrenals from patients who have died at different intervals after conditions of severe stress, such as coronary thrombosis and severe trauma, particularly burns. It is assumed that the morphological changes observed result from liberation of endogenous ACTH. Now, as a result of treatment of breast cancer by bilateral adrenalectomy, it is possible to assess the effect of exogenous ACTH on the adrenal cortex by direct methods It is very significant that the morphological changes which occur in the gland as a result of stress or endogenous ACTH action and those which are seen after administration of ACTH prior to adrenalectomy are similar in most respects. The zone significantly altered in both instances is the zona fasciculata, where the large, lipid-laden *clear* cells are converted to lipid-poor, eosinophilic *compact* cells It is proposed to describe the changes seen in the adrenal glands post mortem in normal and stressed patients and then the effect of exogenous ACTH on glands removed at operation

Effect of stress on the human adrenal cortex

Full details of the changes are given elsewhere (Symington *et al.*, 1955). The 'normal' adrenal gland consists of a narrow zona reticularis composed of compact cells, which are poor in lipid and have a pink eosinophilic cytoplasm when stained by haematoxylin and eosin The cells of the zona fasciculata are swollen with large vacuoles of lipid material. When paraffin sections are stained with haematoxylin and eosin, they have a scanty cytoplasm and appear vacuolated Hence the term *clear* cells. When a severely burned patient dies three days after injury, significant changes are seen in the cells of the zona fasciculata. Lipid depletion occurs, the sudanophil vacuoles become smaller and more discrete and

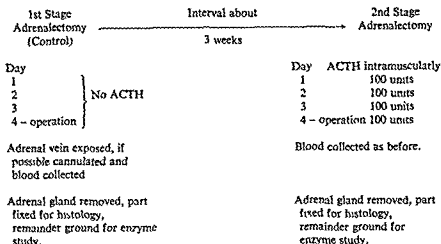


Figure 1.

Table 1

Adrenal response to ACTH (400 units given over a period of 4 days).

Morphology and Histochemistry

	Zona Reticularis		Zona Fasciculata	
	Before ACTH	After ACTH	Before ACTH	After ACTH
Cells	Compact	Compact	Clear	Compact
Cholesterol	Scanty	Scanty	Abundant	Scanty
Acid phosphatase	Abundant	Abundant	Scanty	Abundant
Alkaline phosphatase	Abundant	Abundant	Scanty	Abundant
Succinic dehydrogenase	Moderate	Moderate	Scanty	Abundant
Ribonucleic acid	Abundant	Abundant	Scanty	Abundant

Chemical Analysis of whole Adrenal

	Before ACTH	After ACTH
Ribonucleic acid (RNA-P) (mg /100 g)	13–36	29–54
RNA-P/DNA-P	1 3–2 4	1.8–4 9
11β-hydroxylation (μg corticosterone/mg N/hr	Less than 40 μg	60–180 μg
Cortisol/corticosterone ratio (adrenal effluent)	1.3–2.3/1	3.0–9.8/1

eventually disappear completely from the cell which becomes an eosinophilic compact cell morphologically similar to that of the zona reticularis. In the adult patient lipid depletion occurs in a focal manner and so areas of zona fasciculata rich in lipid alternate with others which are lipid-depleted. The lipid free areas are composed of columns of compact cells and in such areas the customary subdivision into zona reticularis and zone fasciculata is lost. In a few instances (5 %) where the stress is prolonged and severe the glands are completely devoid of lipid, and the cells are exclusively compact in type.

It will be seen that the reaction to stress produces changes in the cells of the zona fasciculata and it is presumed that this is an effect of endogenous liberated ACTH

The reaction of the human adrenal to exogenous ACTH

When patients are subjected to a two-stage bilateral adrenalectomy as shown in Figure 1, the gland removed during the first stage is usually normal in appearance and acts as a control. The second gland removed three weeks later has had time to recover from the first operation and the effect of 400 units of ACTH administered before the second operation can be assessed. Morphological changes in the two glands can be compared with one another and correlated with the 11 β-hydroxylating enzyme content of the glands. Since it has been possible to cannulate the adrenal vein (Grant, Forrest & Symington, 1957) it is also possible to compare the effect of ACTH on the ratio of cortisol to corticosterone in the adrenal effluent.

The changes are shown in Table I and it will be seen that, as in the reaction to stress, the significant effect of exogenous ACTH is on the cells of the zona fasciculata The clear cells, rich in lipid material, contain little stainable alkaline or acid phosphatase, succinic dehydrogenase, ribonucleic acid (RNA) and few mitochondria are seen using Altman's method or by electron microscopy After 400 units of ACTH the cells of the zona fasciculata become compact in type, the cytoplasm is poor in stainable lipid, but rich in alkaline and acid phostphatase, succinic dehydrogenase and other enzymes of the Krebs' cycle RNA becomes abundant, the cells are packed with mitochondria which are prominent on electron microscopic examination (Carr, 1958, 1958). There is an actual increase in cytoplasmic RNA as shown chemically (Symington & Davidsen, 1956). The compact cell of the zona fasciculata after pro-

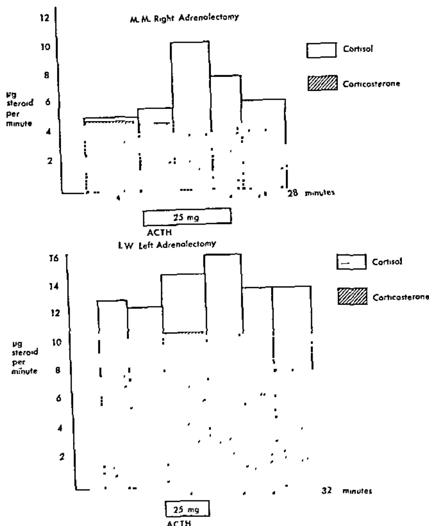


Fig 2

phosphate dehydrogenase enzymes (Studzinski, 1960). However, when ACTH is given to the human patient over a period of days, there is an enhanced output of steroid and an alteration of the cortisol/corticosterone ratio This is associated with morphological and histochemical changes and an increase in the 11 β-hydroxylating enzyme and glucose-6-phosphate dehydrogenase (which increases production of TPNH, a

longed ACTH treatment appears morphologically similar to the compact cell of the zona reticularis.

The histological changes observed in the cells of the zona fasciculata after four days ACTH are associated with a rise in the 11 β-hydroxylating capacity of the gland (Grant, Symington & Duguid, 1957) and a rise in the cortisol/corticosterone ratio from 1–2/1 to 10/1.

Enhancing or priming effect of ACTH on the adrenal cortex

Figure 2 shows the effect of 25 units ACTH injected intravenously into a patient (M.M.) whose adrenal gland was shown to be normal histologically and into another patient (I.W.) whose gland had been stimulated endogenously by an adrenalectomy one week previous. The adrenal pattern in the second patient is one of reversion as described by Sarason (1943) and shows a deposition of lipid in the cells of the inner zona fasciculata This is the mechanism by which the adrenal gland recovers from an operation The steroidogenic reaction of the two glands is different The normal gland produces a rise in both cortisol and corticosterone, while the adrenal which has been endogenously stimulated shows an enhanced production of cortisol.

Thus, an adrenal stimulated endogenously by a previous stress or by 400 units of ACTH over a four-day period shows an enhanced steroidogenic ability with an increase in cortisol/corticosterone ratio in the adrenal vein effluent. Kappas and Gallagher (1955) and Liddle *et al* (1954) demonstrated a progressive stepwise increase in urinary steroids after a daily dose similar to the one used by us Nugent and his colleagues (1959) infused 4 units ACTH over a 24-hour period and observed a rise in plasma 17(OH) corticosteroids from 12 to 42 μg./100 ml When the dose of ACTH infused over 24 hours was cut to 2 units, the plasma 17(OH) corticosteroid level was maintained and remained so during the next 24 hours when the dosage of ACTH was only 1 9 units per day. Immediately the ACTH infusion was stopped the blood corticoids fell, but responded in a very enhanced manner to a 25 unit ACTH *infusion test*

It is well established that ACTH can act on the adrenal gland with rapid production of cortisol and corticosterone within 2 to 8 minutes without altering the cortisol/corticosterone ratio (Bush, 1953) The steroidogenic effect is not associated with any morphological or histochemical changes and no increase in the 11–ß-hydroxylating or glucose-6-

THE ACTION OF CORTICOTROPHIN ON ENZYMES OF THE ADRENAL CORTEX IN MAN

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When we became interested a few years ago in the action of corticotrophin on the adrenal cortex in man, existing knowledge was based almost exclusively on work with animal glands. In particular, the valuable observations of Pincus and his collaborators at the Worcester Foundation in America had been made on the perfused adrenal of the ox—a gland which differs remarkably from that in man, as Professor Symington has pointed out.

The American work resulted in the establishment of the now well known sequence of reactions involved in the biosynthesis of adrenocortical steroids. (Fig. 1) This sequence is in accord with the observation of Bush (1953) that *in vivo* corticotrophin causes a rapid increase in the production of both cortisol (F) and corticosterone (B) in the dog and cat without markedly influencing the F/B ratio, an observation in agreement with the view that both F and B are derived from a common intermediary and that corticotrophin acts at some point in the sequence *before* the production of this common intermediary. The last intermediary common to both F and B is progesterone and this led to the suggestion that corticotrophin must 'activate' some step between cholesterol and pregnenolone, the immediate precursor of progesterone. Some further details of this part of the biosynthetic scheme are shown in Fig. 2.

It should be noted that the degradation of the cholesterol side chain may involve the introduction of hydroxyl groups (Dorfman, 1957) a process known to involve the coenzyme triphosphopyridine nucleotide (TPN) in the reduced state (Halkerston, Eichhorn & Hechter, 1959). The transformation of cholesterol into progesterone probably occurs in the mitochondria of the adrenocortical cells. It is also remarkable that this point of attack of corticotrophin was postulated and widely accepted although it was recognised that the step or steps between cholesterol and progesterone were not specifically related to the adrenal but probably occur in the biosynthetic sequence of events in every steroid producing endo-

cofactor in steroid hydroxylations) (Studzinski, 1960). There is likewise an increase in ribonucleic acid (Symington & Davidson, 1956).

It is possible that the enhanced response to ACTH is linked with the changes observed in the zona fasciculata cells after prolonged ACTH and particularly with the increase in cofactor mechanism, as suggested by Haynes and Berthet (1957). This delayed but enhanced response of the primed adrenal to subsequent ACTH injections is of considerable importance and would appear to throw some light on the enhanced response of the adrenal in Cushing's syndrome to normal amounts of ACTH.

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BIOSYNTHESIS OF C_{21} ADRENOCORTICAL STEROID HORMONES

Fig. 2

ACTH

ADRENAL PHOSPHORYLASE

GLYCOGEN → GLUCOSE-1-PO_4

GLUCOSE-6-PO_4

GLUCOSE-6-PO_4 + TPN + → 6-PHOSPHOGLUCONIC ACID + TPNH

TPNH

CHOLESTEROL → HYDROXYLATED DERIVATIVES OF CHOLESTEROL

PROGESTERONE ← PREGNENOLONE

CORTISOL
CORTICOSTERONE

FIG. 3. MECHANISM OF ACTH ACTION (BASED ON HAYNES AND BERTHET, 1957)

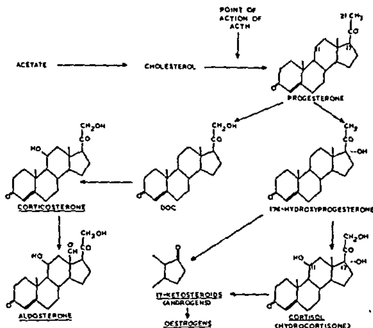


Fig 1

crine gland In the adrenal gland there is the added difficulty that stimulation of this early step might be expected to enhance aldosterone biosynthesis whereas it has little influence on the secretion of this hormone.

Rather large amounts of corticotrophin were used in the perfusion experiments. Maximum effect had been shown with 0 1 unit of corticotrophin; nevertheless 6 units were used Moreover the introduction of the corticotrophin into the perfusion medium did influence the F/B ratio to some extent in so far as the F production increased $\times$ 7 and the B only $\times$ 3 Pincus et al. (1951) had already drawn attention to the fact that increasing F production seemed to inhibit B production.

In contrast with the views of the Pincus group, Heard et al. (1956) put forward the view that corticotrophin activates steroid hydroxylation reactions, in particular the conversion of DOC to what he thought was cortisol and corticosterone Heard's conclusions were, however, unsatisfactory from a variety of considerations It seems likely that the increased conversion of DOC to corticosterone by 11β-hydroxylation could

BIOSYNTHESIS OF C21 ADRENOCORTICAL STEROID HORMONES

Fig 2

ACTH

↓

ADRENAL PHOSPHORYLASE

↓

GLYCOGEN ⟶ GLUCOSE-1-PO_4

↓

GLUCOSE-6-PO_4

GLUCOSE-6-PO_4 + TPN + ⟶ 6-PHOSPHOGLUCONIC ACID + TPNH

TPNH

CHOLESTEROL ⟶ HYDROXYLATED DERIVATIVES OF CHOLESTEROL

PROGESTERONE ⟵ PREGNENOLONE

⟶ CORTISOL, CORTICOSTERONE

FIG. 3. MECHANISM OF ACTH ACTION (BASED ON HAYNES AND BERTHET, 1957)

be explained by an increased rate of penetration of DOC under the influence of the added corticotrophin into the tissue slices used by Heard. (Corticotrophin action has been demonstrated *in vitro* using whole cell preparations but not when the cells are disrupted—as in homogenates). Hechter (1958) has built a general theory around the idea that corticotrophin influences the permeability of cell membranes. I shall return to the question of the influence of corticotrophin on steroid hydroxylation when describing our own work.

The latest view on the influence of corticotrophin on adrenocortical enzymes is that this hormone stimulates an adrenal phosphorylase (Fig. 3) This provides more glucose-6-phosphate, oxidation of which increases the concentration of reduced TPN required for steroid hydroxylations and thus increases hormone production. If this is the case corticotrophin should not be limited in point of attack to the transformation of cholesterol into progesterone but might be expected to stimulate later stages of the biosynthesis which involve hydroxylation. This theory was advanced without any real knowledge of the state of reduction of TPN in adrenal cells. According to Glock & Maclean (1955) TPN is almost entirely in the reduced state in rat liver cells If this is also the case in adrenal cells it is difficult to see the significance of the stimulation of the adrenal phosphorylase.

Haynes (1958) has recently made the very interesting observation

LAST STEP IN SYNTHESIS OF ADRENOCORTICAL STEROID HORMONES

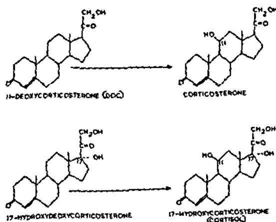


Fig. 4

that corticotrophin causes an increase in the concentration to the unusual adenosine 3', 5'-monophosphate in adrenal slices. This substance is not to be confused with the adenosine monophosphate related to ADP and ATP. The full significance of this discovery remains to be clarified

Koritz & Péron (1958) found that both (a) corticotrophin and (b) TPN reducing systems (e.g. TPN + glucose 6-phosphate + G6P dehydrogenase) stimulate the production of corticosteroids by adrenal slices and that the effects of (a) and (b) are not additive suggesting that the action of corticotrophin on steroid biogenesis is not limited to a stimulation of TPNH production. Alternatively the corticotrophin may influence precursor level—e g. by making mitochondrial cholesterol available. I shall return to the question of multiple action of corticotrophin at a later point.

Our own investigations began with a study of the action of corticotrophin administered *in vivo* on the ability of the human adrenal gland to effect the last step of steroid hormone biogenesis, 11β-hydroxylation (Fig. 4). Glands were obtained from patients undergoing bilateral adrenalectomy for treatment of carcinoma of the breast. One gland was removed without administration of corticotrophin or other hormone, the other after giving corticotrophin (Fig. 5). In all cases administration of corticotrophin resulted in an increase in 11 β-hydroxylation by the ground (homogenised) glands and replacement of the lipid rich 'clear' cells of

PLAN OF INVESTIGATION.

1st STAGE ADRENALECTOMY (CONTROL)	Interval about 3 weeks →	2nd STAGE ADRENALECTOMY
Day. 1, 2, 3, 4-operation — No ACTH		Day. ACTH intramuscularly. 1 — 100 units; 2 — 100 units.; 3 — 100 units; 4-operation — 100 units
Adrenal vein exposed, if possible cannulated and blood collected.		Blood collected as before
Adrenal gland removed, part fixed for histology, remainder ground for enzyme study.		Adrenal gland removed, part fixed for histology, remainder ground for enzyme study.

Fig. 5

ASSESSMENT OF HUMAN ADRENAL PATTERNS.

Adrenal Pattern.		*Assessment.*
"Normal" Lipid-Laden Adrenal		0
Focal lipid depletion	1–25 % of Cortex	+
	25–50 % of Cortex	++
	50–90 % of Cortex	+++
Almost complete or extreme diffuse lipid depletion.		++++

Fig 6

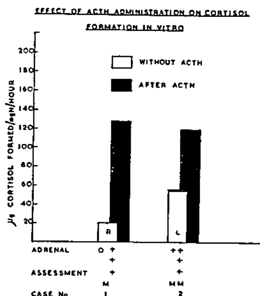
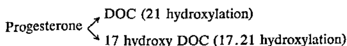


Fig 7

the fascicular type by the small 'compact' cells of the reticular type. Indeed the histological assessment of the glands correlates well with the extent of 11β-hydroxylation (Figs 6, and 7). The effect of corticotropin administered *in vivo* on hydroxylation at the 17 and 21 positions was measured by following the reactions.



using human adrenal homogenates centrifuged to remove nuclei and mitochondria (11β-hydroxylation is confined to the mitochondria) The results expressed as ratios of the amounts of DOC and 17-hydroxy-

Table 1.

Effect of in vivo administered corticotrophin on steroid 21 and 17 hydroxylation in vitro.

Lipid depletion	*DOC/17 hydroxy DOC*
0 to 1+	2 1, 3 1, 2.1, 2·1
3+ to 4+	1 3, 1:1, 2 5 1, 1·1, 1.1, 1.1.5, 1:2

DOC formed are given in Table 1. The corticotrophin action was assessed as degree of lipid depletion, 0 to 1 + indicating no exogenous hormone treatment, 3 + to 4 +· extensive hormone action. It may be seen that corticotrophin tends to increase 17-hydroxy DOC formation. This may possibly be due to an increased activity of the 17-hydroxylating enzyme. Hydroxylations at 11, 17 and 21 all require TPNH and the preferential stimulation of 11 and 17 hydroxylation by corticotrophin adds weight to the belief that the action of this hormone is not limited to stimulation of TPNH production.

Collection of adrenal venous blood from some of the patients by Mr A. P. M Forrest revealed that prior administration of corticotrophin results in an increase in rate of blood flow (Grant *et al* 1957) and an increase in cortisol output per minute The corticosterone output actually decreased (Fig 8) in keeping with the suggestion of Pincus *et al.* (1951) that hydrocortisone inhibits corticosterone biosynthesis.

CORTICOSTEROIDS IN HUMAN ADRENAL VENOUS BLOOD

BLOOD FLOW ml/min.	CORTISOL ug/ml.	CORTISOL ug/min.	CORTICOSTERONE ug/ml.	CORTICOSTERONE ug/min.	HISTOLOGICAL ASSESSMENT
			AFTER ACTH		
–	7 0	–	0 60	–	+ + +
18	3 2	5 8	0 33	0 59	+ + +
2 7	1 5	4 1	0	0	+ +
2·0	3 8	7 6	0 66	1 32	
			WITHOUT ACTH		
1 25	2 8	3 5	2 3	2 87	+

Fig 8

ASSESSMENT OF HUMAN ADRENAL PATTERNS.

Adrenal Pattern		*Assessment.*
"Normal" Lipid-Laden Adrenal		0
Focal lipid depletion	1–25 % of Cortex	+
	25–50 % of Cortex	++
	50–90 % of Cortex	+++
Almost complete or extreme diffuse lipid depletion		++++

Fig 6

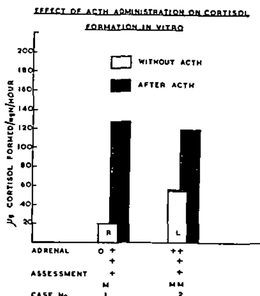
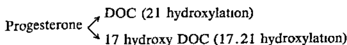


Fig 7

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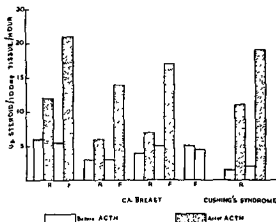


Fig 10

Table 2

Biosynthesis of steroid by human adrenal tissue slices in vitro.

ACTH	Cortisol	Steroid as µg/100 mg tissue/hour	
		Cortisone	Corticosterone
	Zona Reticularis		
−	2 5, 1 88	0, 0	2 2, 0 4
+	17.8, 3 90	3, 0	9 7, 1 2
	Zona Fasciculata		
−	3 6, 3.8	0, 0	2.7, 1 5
+	34 0, 14 9	3, 2	6 4, 8 1
Case of Cushing's Syndrome			
	Zona Reticularis		
−	1 4	0	0 86
+	24 2	0	3 80
	Zona Fasciculata		
−	2 9	0	1 0
+	42 0	0	5 0

The significance of these results is not clear at present and is under investigation.

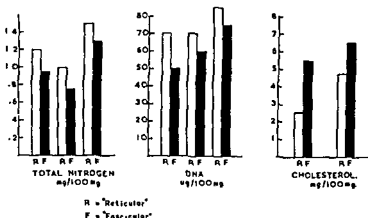


Fig. 9

Since increased cortisol biosynthesis appears to accompany an increase in the number of compact (reticular zone) cells, it seems possible that this cell is primarily concerned with biosynthesis while the lipid rich fasciculata cell is merely a storage cell In order to investigate this, my colleague Mr. Griffiths separated slices of the reticularis and fasciculata of human adrenal cortex. The identity of the zone was established by histology, total nitrogen, DNA and cholesterol determinations (Fig. 9) As might be expected the lipid laden fascicular zone is relatively low in nitrogen and DNA but rich in cholesterol

To our surprise we found that homogenates of both zones showed an equal ability to effect 11β-hydroxylation *in vitro*. Hence the increased 11β-hydroxylation in the corticotrophin stimulated glands cannot be attributed to the increase in the number of compact cells After corticotrophin homogenates of both zones showed an increase in 11β-hydroxylating activity and thus the fasciculata cannot be regarded merely as a storage zone.

We next turned our attention to the short term action of corticotrophin *in vitro* on reticular and fascicular zones We used the technique of Saffran & Schally (1955) The results are shown in Fig 10 from which it may be seen that 0 2 units of corticotrophin cause a more marked stimulation of corticoid secretion from the fascicular than from the reticular zone slices. The effect was especially pronounced with slices from a gland of a patient with Cushing's Syndrome. When the individual steroids were determined separately by paper chromatography and blue

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DISCUSSION FOLLOWING THE PAPERS BY PROFESSOR SYMINGTON AND DR GRANT

Dr. Beryl Davies: These were studies on human blood ACTH Blood was collected straigth into acetic acid and then extracted by the oxycellulose procedure. It is a small series One sample of normal blood was taken and pooled from three normal volunteers and one hypertensive patient who did not have Cushing's disease and two Cushing's patients After the extractions the ACTH concentrates were bio-assayed using hydrocortisone treated rats and intravenous injection of the ACTH The results were that the normal value was 0 8 milli-units per 100 ml, the hypertensive value was 0 72, thus proving that any differences in Cushing's cases were not attributable simply to hypertension, and the mean of two Cushing's patients was an increase of 2½ times in the concentration of ACTH in the blood I am not sure that I have made it quite clear that these were milli-units of ACTH per 100 ml of blood 0 8 in the normal, 0 72 in the hypertension, 2 04 in one Cushing's case and 1 78 in the other Cushing's This increase of ACTH concentration in the Cushing's ties in with Professor Symington's suggestion that they have a primed gland We hope to continue this work so that we can make some correlation between the levels of blood ACTH and the clinical picture of the Cushing's cases Of the two that we are reporting now, one patient died and the relatives refused a post-mortem. In the second case an adrenal gland was removed and it was hyperplastic There was not a tumour on it We also hope to do repeated ACTH estimations on a single Cushing's case to see if there is any variation from day to day.

Dr. Tait: Farrell, sometime ago, reported that in the dog he measured the dexycorticosterone, compound S, F & B, in the hypophysectomized dog and also I believe the ACTH treated hypophysectomized dog—the ratio of the 11-deoxy to the 11-hydroxy compound was fairly constant and this seemed to argue, in that species at any rate, against ACTH acting dominantly upon 11-hydroxylation I wonder if Dr Grant had measured compound S, difficult though this task would be, in the human adrenal venous blood

Dr. Grant: We thought about this because—was it not Touchstone's group that did quite a bit of work on compound S?—we were concerned whether what we were measuring as corticosterone was in fact corticosterone and not a

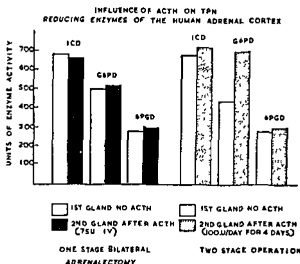


Fig 11

tetrazolium colour reaction it was found that the most pronounced effect was on cortisol production (Table 2). It is well known that these steroids are not stored in the gland and thus values shown represent newly synthesized steroid.

Dr. G Studzinski has investigated the effect of corticotrophin on a variety of dehydrogenases known to be coupled with TPN reduction. His results are summarized in Fig. 11 from which it is quite clear that *the most striking increase in activity of these enzymes (ICD = isocitric* dehydrogenase, G6PD = glucose-6-phosphate dehydrogenase, 6PGD = 6-phosphogluconic acid dehydrogenase) is observed when corticotrophin has been administered for some time, rather than when the gland is briefly exposed to corticotrophin given intravenously during the operation There is good evidence however that this brief exposure to corticotrophin results in an increased corticoid production This is further evidence in favour of the idea that at least two mechanisms of action of corticotrophin can be distinguished.

animals which are ruminants have this type of gland filled up with compact cells. All the rest, the dog, man, monkey and so on, all the non-ruminants, have got the two zones, the fasciculata and reticularis As Professor Symington says the bovine adrenal looks like a very heavily stressed adrenal, a primed adrenal. It has got a cortex full of these compact cells.

Dr. Dixon: But it is not strange that the primed adrenal gets more compact cells and becomes more responsive to ACTH and yet when slices of compact and clear are compared it is the clear cells that are more responsive to ACTH?

Prof. Symington: I think that this is an important point If it is overprimed and you lose all the lipid, then it won't respond to ACTH I think that is what Hechter did find. His ox gland responded very much better to added cholesterol than *either* to ACTH or to progesterone. It responded to progesterone without ACTH but he did not get an increased response, when he added progesterone and ACTH, comparable to what he did get when he added cholesterol. If you do deplete all the lipid then your gland won't respond to ACTH, unles you add cholesterol

Dr. Cope: I am getting confused here I was under the impression that we got to the point where the lipoid depleted gland was the one that was maximally stimulated, by ACTH, and therefore producing the maximal amount of steroid Now you are suggesting that when it is lipoid depleted it ceases to be able to produce

Prof. Symington: No No

Dr. Cope: That is bringing back the old idea of adrenal exhaustion

Prof. Symington: We have never really got an absolutely depleted gland with ACTH. Even in the ones I did show there was *some* lipid material

Dr. Cope. It is not likely to go suddenly from a state of maximum production to a state of hypo-production when the last granule of lipid disappears, is it?

Prof. Symington· No Not at all The type of gland which is completely depleted of lipid will not respond to ACTH to the same extent. I think that that was the thing that Hechter did show I he took the ox gland and he added cholesterol to that gland and then added ACTH he got a very marked conversion If he added progesterone to the gland he got a conversion but he got no increase if he added ACTH.

Prof. Stuart-Harris: But surely if what you say is applicable to man, how do you account for this increase in output as you continue ACTH stimulation, because would not the depletion be occurring during this early stage?

Prof. Symington: It is being depleted during the early stage but you have still got cholesterol available I don't know what happened to that child s gland if there is no lipid material there It could still form steroid as Hechter showed in his ox gland Does it form its own acetate? It seems to me that Hechter was right In the initial stage is the conversion of cholesterol to progesterone If you have got cholesterol there you can utilize it and utilize it quickly

Dr. Cope: Is there any suggestion, or any evidence, maintaining the old view that you could get exhaustion of the adrenal?

Prof. Symington: No we are not implying exhaustion. I don't think that you do get exhaustion at all I think that if you completelly deplete the lipid you are not working with a gland that has got tremendous potential It can still I am sure, form steroid If you find these completely depleted glands at post-mortem and do a blood steroid assay you get a high normal result. I don't know how it forms it

Prof. Graham Wilson: The difficulty about this for us, who are unitiated in this is this concept of a lipid depleted gland. I cannot quite follow this, I am

mixture of corticosterone and compound S As you know they run together on most chromatograms One can distinguish them by doing a different type of reaction. We had been measuring our steroids from the adrenal venous blood by the so-called blue tetrazolium reaction, but there is a more specific reaction for compounds having a hydroxyl group at the 17 position—at which corticosterone has no hydroxyl—namely the Porter-Silber reaction In the adrenal venous blood samples that we collected afterwards, we did a Porter-Silber reaction and found that the amount of Porter-Silber chromogen was not significantly greater than the blank and what we were calling corticosterone was, in these cases at any rate, corticosterone I think that Touchstone's observations, where he got relatively high amounts of compound S, were in hypertensive patients Patients with malignant hypertension who had undergone adrenalectomy and from whom he was able to collect some adrenal venous blood I would not say this of all our cases, because we did not check this until somewhat later, but we feel fairly sure that in the cancer cases, although there may have been a small amount of compound S contaminating what we called the corticosterone fraction, it *was* predominantly corticosterone.

Dr. Tait: I was just wondering whether you could measure corticosterone in human adrenal venous blood by using a labelled reagent method or something like that which would be very sensitive—would not this be a rather crucial test for the theory of ACTH action upon 11-hydroxylation?

Dr. Grant: Yes

Dr. Tait: As Farrell tested it in the dog

Dr. Grant: Yes I think that you are quite right there

Dr. Norymberski: Some years ago we made a few observations which I think would very nicely fit with what Professor Symington and Dr Grant have demonstrated, namely that in the stressed adrenal there is a more efficient action of the 17 than of the 21 hydroxylase This was observed on a few patients of Professor Jepson's who were treated with ACTH before and after surgery We have measured the 21 deoxyketols which measure metabolites of 17 hydroxy-progesterone and also of 17-11 dihydroxy-progesterone We found after operation this group of compounds responded to ACTH much much more strongly Much larger quantities were excreted in the urine after the certain stimulation of the adrenals

Dr. Grant: The three hydroxylases of progesterone 11, 17 & 21 are all stimulated by reduced TPN and if the mode of action of corticotropin, as according to current theories, is merely to increase the amount of reduced TPN why is it that 21 hydroxylation is not increased? One feels that ACTH has got many actions and I think, as somebody said this morning, we do not need to commit ourselves to the view that every action of ACTH is concerned with the production of adrenal corticosteroids

Prof. Symington: I wonder, Dr Tait, if Dr Hechter thought that in the bovine gland he was really dealing with a primed gland? I think that bovine adrenals show the highest activity of any in glucose 6 phosphate dehydrogenase I wonder whether he had considered this? Because he was really dealing with a gland which theoretically was a primed gland To this he added his cholesterol with very marked conversion

Dr. Grant: I don't know whether this has any real bearing on it but Dr Currie has told me that the content of ACTH in the bovine pituitaries, is higher than in pituitaries of other species You see we divide the adrenals into two types, the bovine type and the non-ruminant type and oddly enough there is one exception to this, (we have looked at a great many sections produced by the pathologist at the zoological gardens in Edinbourgh) the little golden hamster—All the

Prof. Symington: I think that in-vivo, when we stimulate the gland, we get the clear to the compact change and there is an immediate increase in the glucose 6 phosphate dehydrogenase We accept Haines concept that we are building up In the clear cell we have available precursor.

Dr Grant: I think that Dr Dixon has got a very good point there Professor Symington mentioned it He has tried in organ culture to convert fasciculata type clear cells to compact cells but he was not able to do it It rather suggests that there is a blood factor coming into it

Prof. Symington: I don't know what it is but we can't get this conversion even with four days ACTH treatment in organ culture

Dr. Dixon: Do you think that there is any relation between the breaking up into villi of the cell boundary with ACTH and the similar kind of break up, also seen electronmicroscopically, of the adipose-tissue cell with insulin

Prof. Symington: Well that is referred to as pinocytosis This is rather a reverse pinocytosis That invaginates into the cell, this goes out Is it to absorb material into the cell or to let material get out?

Dr. Grant: You could see on some of Dr. Carr's electronmicrographs that these microvilli are channeled right out of the cytoplasm into the clear inter cellular spaces At one time we thought that in this was related, somehow or other, to a mechanism of secretion, but it is very difficult to correlate electromicrographs to function

Prof. Stuart-Harris: I wonder whether, Professor Symington, you have made any observations on adrenal glands from patients who have been dosed heavily with steroids, such as prednisolone and so forth In other words whether you had been able to get any morphological studies of the converse state of affairs Have you got anything to say on that?

Prof. Symington: We haven't.

Mr. Forrest: We have only recently had a chance of doing adrenal cannulations during adrenalectomies on patients after pituitary implantations and after hypophysectomy. More recently we have been trying to get rid of the endogenous ACTH stimulation in the patients by giving them dexamethasone, but these results just aren't through yet There was one hypophysectomized patient who was very interesting She had been hypophysectomized three years previously and had been on cortisone 50 mg a day for these three years She then came to adrenalectomy When she eventually died the hypophysectomy was found to be complete, so she *was* completely hypophysectiomized Despite this her adrenal cannulation was done without any additional steroid, only her 50 mg of cortisone a day Her adrenal venous effluent contained 14 8 gamma of cortisol per 100 ml, whereas her peripheral blood had under 5 so this adrenal was still producing steroid despite the fact that she was completely hypophysectomized Then Dr. Grant did the 11-beta-hydroxylation Was it in the region of 29 or 30 or thereabouts?

Dr. Grant. It was lowish

Mr. Forrest: Yes but her adrenal was in fact still ticking over in spite of the fact that it was entirely without endogenous ACTH

Prof Symington: But we have not done anything on high dosage of hydrocortisone

Prof. Stuart-Harris· That was what I really meant I was interested in this point that you made about the increase in blood flow in these adrenals following ACTH because of a particular patient. We had one, among several we knew of who had died suddenly following withdrawal of steroids by their own family doctors, who came to autopsy She had adrenal haemorrhage in what should have been an atrophied gland one would have imagined

afraid You say that you cannot show histologically a completely lipid depleted gland That is material taken from a living animal, not a slice or something like that, so presumably the rate limiting factor is *not* the ability of the gland to take up cholesterol from the blood stream. There must be always cholesterol getting in from the blood This is my difficulty in following this at the moment

Dr. Cope: Can we take the view—I am trying to simplify this—that the situation is getting to a stage comparable to the thyroid where colloid, as an evidence of inactivity, is comparable to the lipoid, as evidence of inactivity in the adrenal gland

Prof Symington: I am not quite sure that the comparison is correct I think that we accept that the human gland has a reticularis that on the Saffran assay can form steroid, and that it has a fasciculata with precursor which is very responsive to ACTH and reacts with a sudden outburst of steroid Now if we deplete the lipid, using exogenous ACTH, and get to the stage that we got to in the child, the burnt child, of having a completely lipid depleted gland, if we could get to that stage, *then* we would be in a position to see whether or not, or what, it is producing But we have never really got to that stage Hechter, I think, got nearest with his bovine gland If he added cholesterol to that bovine gland and perfused ACTH he would get a marked response Now that is what I would call, theoretically, the exhausted gland If you give it something to work on it will work. But if it gets no cholesterol, can it do it from something else? I don't know

Dr. Grant: We rather thought that bovines might be different because they might have short chain fatty acids or lipids of some sort coming from their *rumen In fact the bovine is making its adreno-cortical steroids from something* it gets from its blood stream because it has virtually no store of cholesterol I don't know whether we have made it clear about this lipid depletion but I think that what Professor Symington means and what I understand by lipid depletion is this movement out through the cortex, a kind of sweeping through of the cells, the clear fascicular zone cells disappearing They lose their lipid, and are replaced by compact reticular zone type cells which are free from gross lipid that you can stain histologically But we have never, in prolonged administration of ACTH to women with breast cancer before adrenalectomy, got a picture in which there have been no clear cells at all There are always a few clear cells, that's what we mean when we say that we never get a completely lipid depleted gland

Dr. Fotherby: Does the stain you are using react with the cholesterol esters or just with the free cholesterol?

Dr. Grant: I think the free cholesterol

Dr. Fotherby: So there could be esterified cholesterol there available for de-esterification and conversion to hormone

Dr. Grant: That would take up Sudan wouldn't it—esterified cholesterol?

Prof. Symington: It would

Dr. Stack-Dunne: *I think that we have noted with the rat* adrenals that the cholesterol content is often a lot more than you would think from the histologically visible lipid A lot of that lipid is probably neutral

Prof. Symington: Yes I think that that is reasonable

Dr. Dixon: You find, as I understand, that the gland as it gets more compact cells becomes more responsive to ACTH in-vivo—the build up Also when you cut the slices dividing the clear from the compact it is, on the other hand the clear that respond better Could that be that in-vivo you have got the precursors available and therefore the compact zone can really respond better, but in-vitro cannot because the precursors are not arriving?

Prof Symington: We had one that did not respond at all

Dr. Grant: Yes that was right The adenoma was one of those massive yellow lipid infiltrated types of adenoma It did not respond at all

Prof. Symington: The question about the histology Some areas look adenomatous but most of the tumour that we got was carcinoma I think that it was really a carcinoma We have never had the opportunity of doing a functioning adenoma

Dr. James: I noticed, when doing some urinary steroid studies after ACTH, that some patients with Addison's disease did not respond to ACTH for about two days, and then responded with a fall in urinary steroid excretion Patients with hyperplasia, the Cushing's patients, have done the same thing I am wondering what Professor Symington thinks is happening to the gland there I find it a little difficult to understand why suddenly get this apparent fall off in production from the gland although you continue stimulation with ACTH

Prof. Symington: I can't tell you But one thing that we can say is that we have three cases of apparent spontaneous remission from Cushing's disease. We have two glands from those cases One of them had fairly severe osteoporosis They had bilateral adrenalectomies The interesting point was that the gland—the reticularis—had been replaced by fatty material. If you do an ACTH infusion in the state you describe what do you get? Have you tried an ACTH infusion?

Dr. James: No it was intramuscular ACTH that we gave.

Prof. Symington. I think that it would be useful to give an intravenous ACTH infusion and see the reaction

Dr Singer: Hasn't there been some work showing that there is a variation, a cyclic variation, in the excretion of steroids in Cushing's syndrome You may just be coming in on a cycle

Dr. James: Yes that is possible, but we followed these patients for a long time and their steroid excretion dropped to a larger extent than it does in this variation I don't see how you could explain *Addison's* patients that way

Dr. Cope: What is dropping in the Addison's patient? Is there any steroid there?

Dr. James. Yes, these are patients who excrete about 6 or 7 mg of ketogenic steroids, but after two days of no response it falls

Prof. Graham Wilson: Are the estimations reliable at that level Can you be certain of the changes at the bottom of the scale as it were?

Dr. James: These are fairly consistent if you do them day after day

Dr. Shuster: We have had a very similar thing in measuring plasma steroids in one patient with Addison's disease He arrived in adrenal crisis with a basal level of plasma 17-hydroxycorticosteroid of 7 or 8 Then after two days of ACTH, 120 units intramuscularly, the plasma level fell to 5 and the urine assays were in parallel

Dr. Norymberski: Birke, Plantin and Diczfalucy did some statistical analyses of about 600 duplicate estimations of 17-hydroxycorticosteroids and on an excretion level between 0 to 5 mg per day they found that the standard deviation, or rather the co-efficient of variation, was more than 20 %, and they finally concluded that under 2 mg the determined values were meaningsless. At least by the existing method

Prof. Stuart-Harris: Are there any more questions?

Dr. Chalmers: There is just one point about the levels of ACTH in the blood in the Cushing's patients I wondered whether you would care to comment on the previous observations which have not shown a raised level.

Dr. Beryl Davies: Yes my reading of the literature is that most of the testing

Prof. Symington: It is not uncommon to find in ACTH stimulated adrenals a *medullary haemorrhage.*

Mr. Forrest: This is most striking at operation. I must have seen well over 150 adrenals now in various states, e. g the hypophysectomized adrenal, the cortisone treated adrenal, the acute and the chronically ACTH treated adrenal The adrenal that is unstimulated is golden yellow in colour, leaf-like and small and the blood flow relatively poor, the blood coming from the vein relatively dark and venous in nature. In the ACTH stimulated gland, the whole gland becomes swollen, becomes purplish, quite a different colour and the adrenal blood flow is more rapid and the adrenal blood is very much better oxygated—the venous blood is—suggesting that there is a bigger flow of blood through the gland This is quite a striking effect.

Prof. Stuart-Harris: Has any one reported instances of actual haemorrhage into the adrenal, following ACTH?

Prof. Symington: I know only of reported cases—one or two

Prof. Stuart-Harris: There have been one or two? In the case I was talking about there was no question of exogenous ACTH It is a case I suppose of taking the brake off as it were The doctor stops the oral treatment and the patient's own pituitary suddenly produces an outflow of ACTH

Prof. Symington: Dr Cope, did not you suggest, that after cortisone treatment for a length of time, even up to two years, certain patients' adrenals will not respond?

Dr. Cope: That is so I don't honestly know the evidence for it. It is generally accepted I think Whether it is in fact as long as that I would like to be more certain

Prof. Graham Wilson: I think that it is a very variable phenomenon I will show you some slides tomorrow It is extremely variable, the suppression you get, after long term cortisone or prednisolone therapy

Prof. Symington: What was the condition?

Prof. Stuart-Harris: I don't actually know I think that it was an arthritis The variability is the important thing Although this may not be perhaps of any academic significance it is of very great practical importance because at the present time, as you know, it is possible for the family doctor to prescribe steroids and many of them are quite unaware of the hazards to patients of leaving off suddenly, when they have been on long term treatment, or even short term treatment with big doses I feel myself that we may well evolve, in relation shall we say to a state such as asthma, much more to short courses of treatment in which intensive dosage is used, and we really need guidance on the clinical side on this particular point as to what we should do Whether we should use ACTH in preference to steroids

Dr. West: There is a move, Professor Stuart-Harris, to have a collaborative study throughout the country, on this problem A meeting is to be held shortly at the Ciba Foundation to devise some way of finding out just what you want to know How to deal with these people—how to be sure that their pituitaries have woken up again as well as their adrenals—how to diagnose in advance that you need to give such treatment and so on

Dr. Stowers: Could I ask Dr Grant, of the patients with Cushings syndrome that had an increased output of steroids in-vitro, were these people with hyperplasia or with adenomas?

Dr. Grant: With hyperplasia

Dr. Stowers: Have you tried any adenoma tissue to see whether it responded to ACTH?

these enormous differences of opinion among people who have used only the crude extract

Dr. Paulsen: To the point Dr West raised I can only say that there is a certain amount of ACTH, both in the posterior lobe and in the anterior lobe, that is not adsorbed to oxycellulose even with very often repeated oxycellulose adsorption This ACTH appears to have a smaller molecule size than the oxycellulose adsorbed ACTH This non-adsorbable ACTH seems to appear in considerable quantities in the posterior lobe.

Prof. Stuart-Harris: Well, I think that we should break off at this point and have some tea.

has been very inadequate on the biological side It has been a question of 2 or 3 rats and *one* dose level only In our assays there were 30 rats and three doses of standard and three doses of unknown and we were measuring ascorbic acid depletion On that aspect we hope that we can substantiate our work later on with a right down regular assay Most of the conflicting results, as I say, seem to me to be due to lack of adequate bioassay The extraction of ACTH from the blood in our case was with oxycellulose There is the difficulty that the acid acetone method gives very high results for normal blood. I think that the Ciba conference that discussed this under Dr. Lorrain's leadership suggested that if normal values were really high you would get no response to injected ACTH in these people. Dr Clayton's results reported in a proceedings of the Royal Society of Medicine are for very high levels of blood ACTH in Cushing's disease. If I have the figures right they were something like 140 milli-units per 100 ml of blood in some cases This is untreated blood, estimated at one level only. So that my picture of it is that both Dr. Currie's and my results give high levels in Cushing's disease and Dr. Claytons results are even higher

Dr. Cope: Have you studied any cases of Addison's *disease*?

Dr. Beryl Davies: No we haven't

Dr. West: We have not heard anything from Dr Paulsen yet this afternoon I don't know whether he would like to comment on this subject. At lunch time he told me that they had been finding ACTH activity in the posterior lobe of the pituitary and that this was not adsorbed by carboxy cellulose I wondered whether that might affect some of these blood assays, if there is such corticotropin circulating in the blood which is not picked up by the techniques you use to adsorb it

Prof. Stuart-Harris: Dr Paulsen would you like to say something about this?

Dr. Paulsen: Well I cannot say whether there is any connection between this finding and the determination in the blood I think that we should not forget that the determination of any protein hormone in the blood is still an insufficiently investigated problem It is the same with the determination of T S H and the growth hormone in the blood, for example We have to regard them with all sorts of reservations There is a possibility that there is binding of the protein hormones to the blood protein, involving temporary inactivation I wonder whether this should not be investigated before we start determining the ACTH in the blood

Dr. West: I think that it is agreed that the binding of steroids in the blood is dissociated by the most gentle of extraction procedures Do you think that the binding might be stronger if it is with polypeptides?

Dr. Paulsen: I was thinking of an example, which I still think is an unanswered problem, in the determination of protein hormones in the blood That is the enormous quantities of certain gonadotrophic hormones that occur during pregnancy for instance, in some species, whereas in others they do not occur at all It is very difficult to imagine that these enormous amounts of gonadotrophic activity can occur in the blood without affecting the gonads in one way or the other There must be some method of temporary inactivation That, I think, is a problem one should not forget

Dr. Beryl Davies. I have nothing to add on the protein binding side but this method of oxycellulose extraction of blood of Sayers In hypophysectomized rat blood to which he added ACTH he recovered 85 % of the added material in one extraction

Dr. Stack-Dunne: It might be worthwhile pointing out that these discrepancies in blood levels of ACTH also occur when using just a crude extraction technique It is not a difference between the crude extract and the oxycellulose There are

ACQUIRED RESISTANCE TO CORTICOTROPIN

H. F. WEST
Rheumatism Research Centre Sheffield

When crude, pre-COC purified, corticotropin was used, acquired resistance, with or without allergic reactions, was common and serum antibodies were demonstrated by many. When the carboxycellulose adsorbed corticotropin ("highly purified") was introduced, these troubles were thought to be at an end Unfortunately some patients who had become highly sensitized to the crude preparations had anaphylactic reactions also to the C O C. purified I thought this should be overcome by *really* highly purified corticotropin and Drs. Dixon and Stack-Dunne kindly gave me a little corticotropin A_1 and A_2. The A_2 proved to be almost as potent as the A_1 Skin testing the previously sensitized patients showed that they were sensitive to porcine and bovine C.O C corticotropin and not to the porcine A_1. The first slide (fig. 1) shows how a patient who

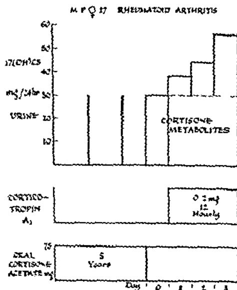


Fig 1

Response of a patient to porcine corticotropin A_1 who had had an anaphylactic reaction to less highly purified corticotropin

loss of effectiveness of the corticotropin cannot be attributed to a change from a potent batch to a less potent one, or to loss of potency due to the improper storage or the age of the preparation. When a number of patients are being treated concurrently with the same batches of corticotropin they will all show the same rises and falls in 17(OH)CS output—*unless* they are developing resistance. Two slides to illustrate this were shown.

Having some more corticotropin A_1 from Dr. Dixon I treated, for a few days, a number of patients who had acquired resistance, without overt allergy, to COC purified corticotropin. They all responded well. I thought this meant that corticotropin A_1 was not antigenic but soon found that a prolonged trial was necessary as acquired resistance, without overt allergy, was soon lost and might take some weeks to redevelop. The next slide (Fig. 3) shows an example of reacquired resistance. The variations up to month 52, with the possible exception of month 22, could be accounted for by variations in batch potency. The loss of effect in months 52–3–4 were to batches of corticotropin which all our other patients (except P.W. of the previous slide) found to be up to, or above, average potency. He reacquired resistance in months 59–62. In the second half of month 59 his 17(OH)CS excretion was 70 mg. on 28 units; in month 62

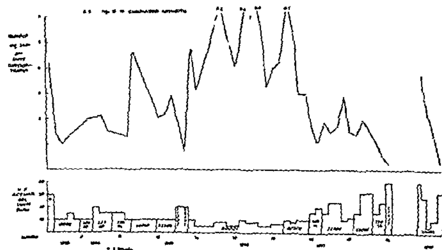


Fig. 3

To illustrate re-acquired resistance (For more detail see Acta Med. Scand. Supplement 352. 1960)

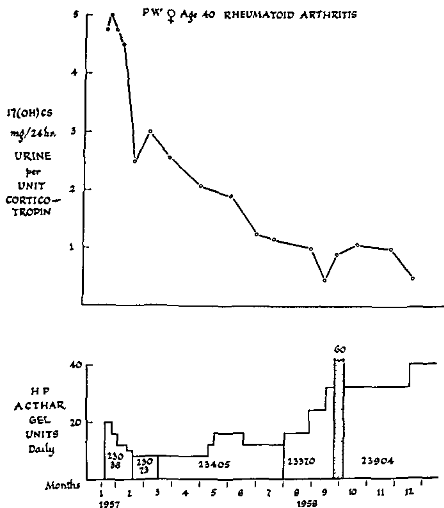


Fig 2
An example of "acquired resistance"

had had an anaphylactic reaction with severe bronchiospasm reacted to A₁. Further slides were shown to illustrate the development of an acquired resistance which *ended* in an anaphylactic reaction This patient also responded, without reaction, to corticotropin A₁ Her serum agglutinated bovine red blood corpuscles while 30 controls did not There were no porcine cells of a suitable blood group available. The next slide (fig. 2) shows a good example of acquired resistance to C.O C. purified corticotropin. Before diagnosing acquired resistance one must be sure that the

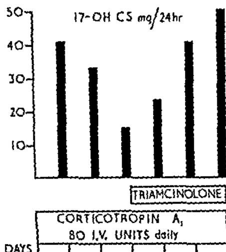


Fig 5

Days 45–50 from fig 4 6 mg of triamcinolone was given daily 80 I V units of A_1 = approx 200 international units

days later. On day 31 she had relapsed so badly that the change to corticotropin A_1 was made. A major clinical response followed but only for a few days. Not until the dose was raised to 80 I.V. units (200 or more ordinary units) was she symptom free again. The next slide (Fig 5) shows what happened next. On day 47 she relapsed so badly that the trial had to be abandoned but before stopping the corticotropin A_1 I added 6 mg daily of triamcinolone I interpret the result—a major remission and a steep rise in 17(OH)CS output—to a partial suppression of whatever process was inactivating the corticotropin A_1. An attempt to demonstrate antibodies in her serum to the corticotropin was unsuccessful—due I think to technical difficulties (see discussion).

The simple explanation that acquired resistance is due to antibody formation may be true in some instances, but the problem is complicated by the fact that some patients may develop an increased sensitivity to corticotropin at some stage in their long term therapy. This was first brought to my notice by Dr. Oswald Savage of the West London Hospital. The next two slides (Fig. 6 & 7) illustrate such occurrences. My conclusion is that acquired resistance is due to antibodies in some patients but that in others the resistance can be easily overcome by raising the dose of

it was 8 mg. on 32 units. To see whether resistance could be acquired, in time, to corticotropin A_1, I treated a patient who had had a generalized urticarial reaction to porcine COC purified corticotropin She responded well for two months until the A_1 supply ran out. (Print of slide not reproduced). Part way through the course of treatment she developed allergic reactions at the site of injection. This was quickly traced to the phenol we had added to the gelatin, as I had myself developed such reactions to a similar preparation. All the other components of the gel were exonerated. At this stage an M R.C. sub-comittee took up the study. Acquired resistance was defined as a failure to respond to double the initial effective dose of a specially prepared COC porcine corticotropin. When this had occured the patient was to be transferred to corticotropin A_1 prepared from the same special batch of corticotropin I chose my most difficult patient She had twice rapidly acquired resistance to COC purified corticotropin The next slide (Fig. 4) illustrates the course of her treatment. Each change of dose up to day 51 was determined by her clinical condition and not by the results of the assays. They arrived some

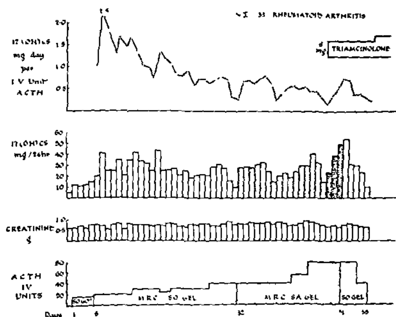


Fig 4

Acquired resistance to corticotropin A_1 S O Gel = C O C purified porcine corticotropin S A Gel = Corticotropin A, prepared from the C O C. corticotropin of S O Gel

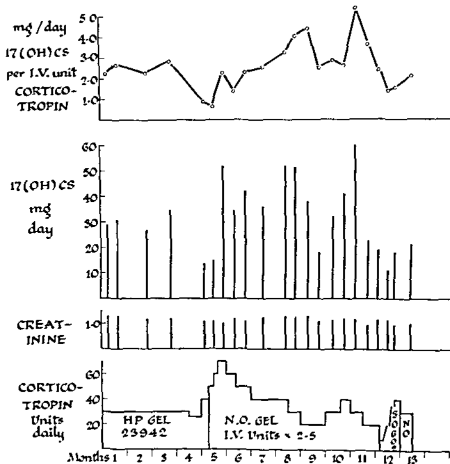


Fig. 7.

To illustrate changes in responsiveness to corticotropin during prolonged administration A factor of 2 5 has been used to convert the I V units of N.O gel to international (subcutaneous) units

a matter of a few months The ACTH was stopped for a period of 6 weeks and then re-started and there was definitely adrenal stimulation when the ACTH was re-started

Dr. James: How often can you inhibit this failure to respond, this reaction to ACTH, by giving exogenous corticosteroids? How often does this experiment work with triamcinolone?

Dr. West: I don't know that it does usually work. In several instances we have had patients on 10 mg. of prednisolone and they have destroyed ACTH while

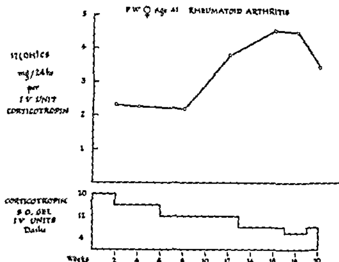


Fig 6.
To illustrate an increase in responsiveness to corticotropin during prolonged administration

corticotropin and it is then due to an alteration or adaptation in whatever the normal destructive mechanism is. Many patients acquired resistance to crude corticotropin, relatively few acquire resistance to COC purified and it may be that resistance to corticotropin A1 will prove to be rare.

DISCUSSION FOLLOWING DR WEST'S PAPER *on ACQUIRED RESISTANCE TO CORTICOTROPIN*

Dr. Paulsen: Could Dr West tell us how common this phenomenon of acquired resistance is? What proportion of patients may show it?

Dr. West: Our cases have all either rheumatoid arthritis or ankylosing spondylitis. I don't know whether the same applies to other diseases. I would say approximately, 1 in 7 og 8 That is patients treated over perhaps a years period When the dose has to go up to 40 units a day we feel that it is uneconomic

Dr. Cope: I was going to raise the very point because one has had the impression, I have had it, that people with rheumatoid arthritis are quite often particularly liable to develop antibodies to all sorts of things. I don't know whether Dr. West would agree with that and that this 1 in 7, or 1 in 8, resistance to corticotropin, seen in the rheumatoid arthritis, may not necessarily reflect the whole picture. It may be much less common in some of the other people who get ACTH

Dr. Fotherby: We have seen it in one case of ulcerative colitis We have only studied three cases so far but one of these has shown an acquired resistance in

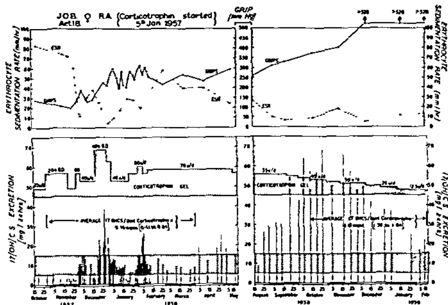


Fig. 2.
See text (Dr. Davis).

dose of the previous preparation and on that particular day his excretion fell right down. After six days it was again in the 90 region. The other slide (Fig. 2) looks rather complex. It illustrates where we disagreed, or appeared to disagree, with Dr. West. We could not come to terms about what we meant by resistance This patient was a girl who started corticotropin in January 1957 with a good initial response After a few months she became resistant, as we have accepted—showing a fall in 17(OH)CS figures and a clinical relapse. She came back into hospital and, to get any response at all, we had to increase her dose to 40 units twice a day. She finally left hospital in a fairly steady state on 70 units a day. This is the interesting thing; after a few months she became spontaneously more sensitive to the corticotropin and, before we knew where we were, we were having to cut her dose right down For the last year she has been carrying on satisfactorily on 10 units a day, with an excretion of 17(OH)CS somewhere round about the 20 region. This is associated with a striking clinical improvement—grip right up and E.S.R. right down Expressing it the other way round—Over the first period the average 17(OH)CS excretion per unit of corticotropin was 0.16 and in the second, a few months later, 1 6—ten times more sensitive The preparations used were all batches of COC purified gel, which were perfectly adequate in other patients at the same time and were of about equal potency There was no period off ACTH to allow her to become responsive again. With continuous treatment, with the same preparation, she seems to have completely overcome her resistance and to have become unusually sensitive From the point of view of the frequency of this, in a series of 45 patients, in whom we have had a 2 year follow up, we have had to stop 8 because of aquired resistance That is with taking them up to about 60 units a day.

we have maintained that dose. Why in this case triamcinolone in a dose of 6 mg worked I don't know, except that we were using enormous doses of corticotropin, 200 to 250 units a day. Was it just enough to release some of that dose? Generally 10 mg. of prednisolone a day does not stop these patients from developing resistance

Prof. Symington: May I ask a very much more general question and probably expose my ignorance What is the benefit of using ACTH in rheumatoid arthritis compared with the exogenous steroids, which patients can take by mouth?

Dr. West: Well, I think that is tomorrow's subject You can imagine, from the slides I have shown you, the vast amount of work that goes into using ACTH—yet we are still using ACTH Personally today, after 9 years, I would rather put a person on ACTH than anything else.

Dr. Davis: Sir, can I say a word or two about our experience, because there was a little disagreement in the early days on this question I would, if I may, show a couple of slides The first (Fig 1) is a good illustration of the response to corticotropin A_1 in patients who have become resistant to the COC preparation This was a patient who had had a very short course of ACTH in another hospital before he came to us. A man with active rheumatoid arthritis He started on an ordinary sort of dose, of a potent batch and did not respond at all, in 17(OH)CS excretion, as you see We then put him on to 40 I.V. units a day of the M R C C.O C. purified preparation. Again there was not a significant rise He then went onto the same dose of A_1 prepared from the COC batch and his excretion of 17(OH)CS went up One day, in error, he was given another

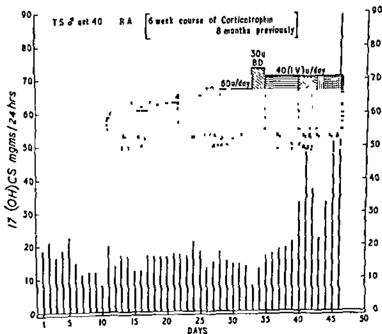


Fig 1
See text (Dr Davis)

Dr. Stowers: I think that it has been shown that so called 'insulinases' destroy ACTH

Dr. Cope: Is this resistance that we are talking about from intramuscular injections. Were they all gel preparations?

Dr. West: Yes

Dr. Cates: You have not found out whether a patient who seems to be resistant to the gel is resistant to an intravenous drip.

Dr. West: We did do this once I do not like giving intravenous drips to people who have developed resistance in case anything goes wrong, but Wolfson, in Detroit, assures me that every patient he has had who has developed resistance, it has been overcome by intravenous injection.

Dr. Cates: Was overcome by it?

Dr. West: Yes, that is to say that they responded

Dr. Cope: Now there are some techniques, I am no immunologist, whereby you deliberately provoke immunity by adding various adjuvants Will gelatin act as an adjuvant? May it be the gelatin that is the nigger in the wood pile here? That is what Cates, I think, is thinking of

Dr. Tindall: You get the same trouble, from time to time, with preparations containing zinc and no gelatin

Dr. Jones: The gelatin itself is non-antigenic. It is tested before us

Dr. Cope: No, but I don't think that these adjuvants are either

Prof. Stuart-Harris: I don't know for certain whether gelatin would act as an adjuvant I would have thought almost certainly not. Because people have been very active in the search for a really good adjuvant preparation I don't seem to have read anything about gelatin I think that something might be said, perhaps, about the manner in which the thing is dispersed Would that be of any significance in relation to this? How is the ACTH dispersed in the gel? Is it in the form of a solution, is it a precipitate or what?

Dr. Stack-Dunne. I think that we might look at this more It looks as if it is in solution but few of these preparations are absolutely clear They are Seitz filtered, aren't they, before use?

Dr. Jones: Yes

Dr. Stack-Dunne: So that if there are particles there—unless they associate after Seitz filtration—they are small enough to go through the Seitz. There could be association

Prof. Stuart-Harris: Adjuvants only work in general when some form of emulsion is produced It is not just a question of depoting, there is something else there which is concerned with properties of emulsions as such. You would think that if this is a foreign body reaction—a reaction to a foreign protein—it should not be too difficult to demonstrate antibody. This strikes me—I am no immunologist —as being odd

Dr Cope: What strikes me too is that we are talking about immunity or immune reactions Things do need clarifying a lot before we are going to get much further forward I think. There may be all kinds of immunity involved here In some patients there may be resistance of the gland itself. Some of the patients had been on long term cortico-steroid therapy. One man had been on prednisone, for 2 years I think you said There is also the species sensitivity, and the corticotropin sensitivity, and there are probably other things as well Do not these things need clarifying in each individual case before we know quite what we are talking about?

Dr West: Certainly, but every patient who has been on prednisolone a long time and who comes back onto ACTH responds, to start with, perfectly. I had

Prof. Stuart-Harris: This last case of Dr. Davis raises serious doubts in my mind as to whether this resistance is really an immunological phenomenon. I wonder whether any definite efforts have been made to illustrate the development, or to obtain evidence concerning the formation, of antibodies? Perhaps Dr. Dixon or Dr. Stack-Dunne would remind us what are the essential differences, if they know them, between the A_1 and these other preparations, which might account for the ability to respond to A_1 after having failed to respond to the ordinary gel.

Dr. West: We have been trying to demonstrate antibodies We have someone who has been trying to demonstrate them for us for some years. First by simple *precipitation tests, later by using tanned red cells and most recently by radio*active labelling. The results following the use of ^{131}I labelled corticotropin are not sorted out yet.

I know of no published evidence of anti-bodies having been demonstrated to highly purified preparations but, in conversation with people in the States, I have been told that they have been *demonstrated—antibodies in the serum—to* COC purified cortiocotropin. I have not seen this in print.

Prof. Stuart-Harris: Dr. Dixon or Dr. Stack-Dunne, can you tell us anything about this question of A_1 and possible differences.

Dr. Dixon: I personally don't know of any I mean I don't know what is likely to be operative The oxycellulose (COC)purified should have between 30 & 50 % A_1, some other active components and some inactive components.

Dr. West: May I ask you if there is any reason why one could not argue from insulin to corticotropin. If there is *any reason why, if the one can induce* antibodies, and I think that everyone agrees that one can get antibodies to insulin when using it from a different species, why that should not be carried over to corticotropin? Are they sufficiently similar polypeptides to argue by analogy?

Dr. Stack-Dunne: I am not expert in this field at all. Corticotropin is quite a big peptide with a molecular weight of 2000 odd It would not be surprising if some antibodies could be formed to it. There is really nothing that you can say. It must be observed.

Dr. Cope: Is this all peptide? You say that there are other substances there. I am not quite sure what you are dealing with Are we dealing with all peptides, of which 50 % is corticotropin, or are there proteins present still?

Dr. Stack-Dunne: I think that it is very probable that in the COC preparation there are materials that you would call protein, much larger molecular weights *than* ACTH In the A_1 it is likely that they are virtually all removed The things that are in A_1 may not all be corticotropin, but they are certainly almost all of about that molecular size.

Dr. Grant: Does anyone know anything about the metabolism of corticotropin? Somebody was talking about the ability to recover corticotropin *from rat blood* Were you talking about that Dr Davies?

Dr. Beryl Davies: Yes, but I am afraid I cannot tell you what the preparation added was Whether it was crude extract or oxycellulose concentrate. It was in the middle fifties that it was done, it obviously was not A_1.

Dr. Grant: I just wondered about that rather odd case of the woman who developed the sensibility to it. Was she ceasing to metabolise the stuff so well? Has no work been done at all on the metabolism of *corticotropin? What happens to it?*

Dr. Beryl Davies: There is an often repeated statement about a '5 minute' biological half-life

Dr. Stack-Dunne: Yes, that is so

Dr. Stowers: I think that it has been shown that so called 'insulinases' destroy ACTH.

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Dr. West: Certainly, but every patient who has been on prednisolone a long time and who comes back onto ACTH responds, to start with, perfectly. I had

assumed that these were tissue antibodies, I had done for some time, but with all this new work on insulin, people are now learning how to detect insulin antibodies using radioactive techniques that are very sensitive, it may well be that once they are applied to corticortopin we may find that antibodies are present. It may be that the bulk of the antibody is fixed in the tissue

Dr. Stack-Dunne: When these people treat themselves with corticotropin for a long period of time, do they inject it in the same position in the skin? Could it be a local effect?

Dr. West: It is intramuscular and they go up and down one thigh and up and down the other thigh

Dr. Stack-Dunne: I was wondering if when a resistant patient has been obtained, if injecting it in the arm, for instance, might produce a difference? If it was really a local effect?

Dr. West: Somebody on the continent did describe, in a paper in German I think, that when put in the deltoid it worked I tried this but it did not make any difference

Dr. Shuster: Is there any immunological phenomenon which will explain the increased response to corticotropin after a period of failure to respond?

Prof. Stuart-Harris: There is an immunological negative phase but it is a pretty feeble thing and not generally regarded as respectable.

Voice: This was very positive?

Prof. Stuart-Harris: Yes, but it would be that way round You have got such an extraordinary interplay here between a gland whose secretion will inhibit immunological changes, and the reticulo-endothelial system, or whatever it might be, trying to produce the opposite effect

Dr. West: Yes, in some patients who develop resistance, on a comparatively small dose, if you put the dose up high enough you will get some corticotropin through to the adrenal, the adrenal will produce cortisol and damp down the antigen—antibody reaction, if it is one, and then their adrenals just whiz away—by compound interest They are stimulated excessively

Prof. Stuart-Harris. Have you done any skin test at all, with a water soluble non-gel containing material, to see if you can find any sensitivity in these people?

Dr. West: Nearly all our testing was in saline, using lyophilized ACTH

Prof. Stuart-Harris: These people who are resistant, are they in any way sensitive *do you know?*

Dr. West: None of them have given big reactions except those who have had overt allergy They are the only people who have had large reactions

Dr. Holti: Are these reactions of the immediate type or of the delayed type?

Dr. West: The delayed type They do give an immediate reaction but so do ordinary people Theirs were not extraordinary —(This description was inadequate. The major reactions to skin testing did not appear after a 'delay', they emerged from the immediate reaction (wheal and flare) and gradually increased in size over several *hours—lasting 1–2 or 3 days*)

Prof. Stuart-Harris: This is the skin The anaphylactic reactions would be immediate

Dr. West: Oh yes

Dr. Holti: If in fact this is a hypersensitivity of the immediate type you ought to be able to demonstrate antibodies by passive transfer

Dr. West: Yes, well I think that it is *not*, except in the cases of anaphylaxis which we no longer see

Prof. Stuart-Harris: You would have to distinguish between the resistance and the anaphylaxis, wouldn't you Although you did show one or two slides of people

who had had anaphylactic or urticarial forms of allergic reaction, it has to be dissociated from the phenomenon of resistance doesn't it?

Dr. Davis: On the question of the resistance In the cases we have tried, where we have really put the dose up, we have always been able to get a response. This is not counting people who have developed local reactions or clinical signs of allergy, or anything like that, but straight forward resistance. There was one woman I remember, we had to go up to 80 units twice a day, which is a pretty big dose of a COC preparation, before we got a response

Dr. Cope: I think that Wolfson said that he would not count it as resistance unless he got no response to 200 units 8 hourly.

Dr. West: I wonder whether we could go back to something that was discussed earlier The business of the adrenal weight factor—whether it exists. You know that when we give ACTH, if we give an adequate dose, the output per day of 17(OH)CS will creep up and up (See fig 3) If you give an insufficient dose (to produce this effect) it goes up a little way and stays level I have assumed that this was due to the adrenal enlarging but from Professor Symington's pictures today I wondered whether it could be due to the lipid gradually withdrawing across the cortex and the whole area coming into full operation

Prof. Symington: We are very interested in this in view of the aetiology of Cushing's syndrome and the concept that Giroud put forward of the potentiating action What he called the corticotropin enhancing factor. He quotes Dr Stack-Dunne in this I would like to hear some views on this

Dr Stack-Dunne: I would not like to take any responsibility for a corticotropin enhancing factor We have observed first and Li has confirmed, that growth hormone in the rat vastly potentiates the adrenal weight effect of corticotropin—in

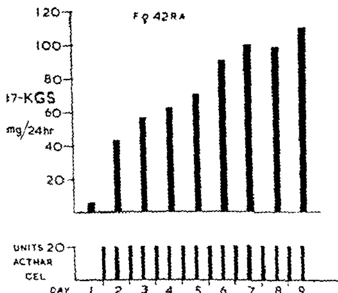


Fig 3
See text (Dr. West)

the hypophysectomized rat. The appearance of the growth hormone synergised adrenal is more nearly normal. It has much lipid in. That is the most distinctive thing, it has a creamier look instead of the purple look. Whether or not this applies to humans I can't say because I have not had an opportunity to observe it in humans and there has been very little growth hormone around, that is active in human It is just possible.

Prof. Symington: What we are interested in is, whether it is necessary to postulate a growth hormone potentiating factor? I think one is getting an enhanced response which every one agrees does occur, in the Cushing's gland to the 6 hour 25 unit infusion of ACTH They are *prepared* apparently and they respond abnormally. There is the high 11-hydroxylation, there is the high 17-hydroxycorticoid and a rather interesting point, Jailer infers that it is not ordinary ACTH because three cases of rheumatoid arthritis, treated with ACTH for 3–5 years, failed to show this response to 25 units of ACTH

Dr. Stack-Dunne: There is no strong reason, that I know of, to connect growth hormone with this All that I can say is that the synergistic action in the rat is dependent upon the dose of the growth hormone, so that if one did have a higher dose of the growth hormone its synergistic effect would be enhanced

Prof. Symington: *We are interested in the relationship* between the growth hormone and the ordinary enhancing effect that we have seen Is it growth hormone or is it something else?

Dr. Stack-Dunne: I think that one would need to do some experiments in hypophysectomized human subject, to see if it is the growth hormone which is active This seems to be the only way to see if there is any relation between our observations and Dr Jailer's theory

Prof. Symington: I wondered if Dr West would comment on this statement that Jailer has made of his three cases of rheumatoid arthritis treated for over *three years with ACTH?* They failed to give this enhanced response to the 6 hour intravenous ACTH infusion. He gives no details of what the ACTH preparations were

Dr. West: I think that the only comment I can make is that we have had one or two patients stimulated much more than we would wish to stimulate them They ran a high output of up to 50 or 60 mg of 17(OH)CS per day for some weeks or months Such people, I find, are very *sensitive to ACTH* and may maintain their high level on a comparatively small dose. I guess that had Jailer's patients' adrenals been stimulated strongly, for some time before the test, they might well have responded to the test as he found Cushing syndrome patients did.

Prof. Symington: They failed to respond That was his point That is why he believes that it is not ordinary ACTH potentiating the gland It must be this growth promoting factor

Dr. West: They may not have had enough ACTH

Prof. Symington: They may not of course There is something else that has to be considered that has been considered in the field before If the ACTH is released in sudden large spurts, perhaps at half hour intervals, it may have a different action on the adrenal over the course of several weeks It is very difficult to assess what *differences you might get* You can well see that is why we were interested in the observations Dr Davies and Dr Currie made of this twice *normal* level of ACTH, correlated with the results of the Samuel's group. It seemed to us to suggest that the potentiating *action could be just* an odd effect of *ordinary ACTH*

Dr. Dixon: The thing in favour of that, I think, is Dr Stack-Dunne's work with growth *hormone* potentiating the steroidogenic effect of acute *ACTH* You did try growth hormone and you got no similar build-up effect with is?

Dr. Stack-Dunne: We tried growth hormone on its own and we tried growth hormone on its own followed by acute ACTH, but we were not able, at that time, to do an experiment on a typically synergised adrenal

Prof. Symington. But if Jailer is correct your growth hormone alone, followed by acute ACTH, should give the potentiating action

Dr. Stack-Dunne: That would be a very abnormal situation because it would be a hypophysectomized rat and there would be a very small adrenal

Dr. Cates: Have any of your rheumatoids died while on a long course of ACTH Dr West, and have you been able to see what their suprarenals looked like? After 2 years or three years treatment

Dr. West: None of our patients have died while on ACTH (On checking this point I found that one patient had died on ACTH therapy—from pneumonia due to coliform organisms that were insensitive to the several antibiotics used His adrenals were reported on as *normal*).

Professor Symington must have seen some

Dr. Davis: We have seen one who was overstimulated and died while in a Cushingoid state His adrenals were large, but not grossly so and from what was seen at histology there was no gross change

Voice: There is a report from Opsahl of Yale that the human placenta contains a lot of ACTH Does anybody really believe this?

Dr. Stack-Dunne: We have looked at the human placenta and found no detectable ACTH, either by intravenous Sayer's assay or by long term peritoneal injection

Dr. West: I would like to get people talking about hypertension and ACTH We have made one or two observations which I think other people could follow up better than we can People have developed hypertension during ACTH therapy in circumstances in which I have thought it could not be due to cortisol

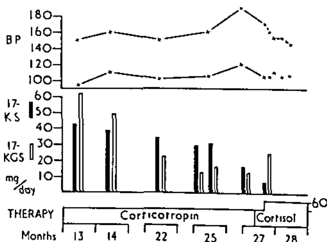


Fig. 4

Fall of 17KGS excretion in 2nd year of corticotropin therapy without fall of 17KS or of "steroid induced" hypertension

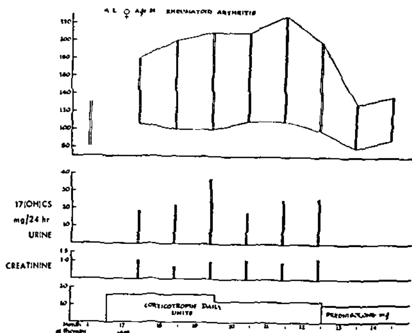


Fig 5.

Hypertension, developing during 22 months of corticotropin therapy, due to the secretion of an adrenal *steroid other than cortisol?*

production I think that everybody knows that people on oral steroid therapy or steroid *therapy however given—with 'glucocorticoids'—and with corticotropin therapy—* some of these patients develop *hypertension*, and others do not We have no clue to why that is On quite high doses of any form of treatment some patients blood pressures will stay absolutely normal In others the blood pressure will rise Some will start with a raised diastolic pressure and it will *not* go on rising, in others *it will* go on rising This is the first patient (Fig 4) that I was suspicious of, way back in 1952–53 He was a case of ankylosing spondylitis In months 13 & 14 of corticotropin therapy you see that his ketosteroids were 40 and his 17KGS 60, but later this ratio was reversed so that after 2 years he had an output of nearly 30 17KS and his 17KGS were down to 12 You see his diastolic blood pressure went up to 110 mm Hg When he was changed to hydrocortisone, 60 mg, there seemed to be a fall The 17KS dropped at once and the 17KGS went up to what you would expect from 60 mg of hydrocortisone I was wondering whether he could have been producing something else there because this ratio had changed I thought perhaps he was producing quite a large amount of corticosteroid which was not cortisol. I thought that it might be corticosterone The next slide (Fig. 5) is from a more recent *patient on corticotropin* The blood pressure began at 130/80, and after 17 months treatment it was above 200/100 You see that when the diastolic pressure was above a 100 the output (17(OH)CS) was only about 20–22 This is quite a low output which I did not think could be responsible for maintaining this hypertension So she was changed

to prednisolone in a dose of 10 mg The clinical effect was equally good, possibly a little better. There was *no* fall in weight to suggest that salt retention had occured on corticotropin and her blood pressure dropped precipitately I suspect again that this patient had been producing something else that was not cortisol When you change from cortisone to prednisolone the blood pressure does not fall. I studied the blood pressure of about 60 patients in a Medical Research Council—Nuffield Foundation clinical trial. They were rheumatoid arthritic patients who had been on cortisone for about two years Half of them were changed, at random, to prednisolone. The mean dose received by each group was equivalent and during the subsequent 12 months the mean diastolic blood pressures did not differ significantly This was so of about 26 of them who had a diastolic blood pressure of 90 or over. The idea that with no salt retention there should not be a hypertensive effect does not seem to work with any of the newer cortisone analogues. Fig. 6 is the record of another patient who did the same thing on changing from ACTH to prednisolone She was clinically very much better and her strenght of grip went right up, but the blood pressure dropped down I suspect again that she was producing something other than cortisol I would very much like to know whether anyone has observed this sort of thing or could look for the steroid (?) that is responsible It might be said of the previous patient that depression caused her blood pressure to fall, because her husband died just about then, but I do not think that it was the case Fig 7 shows a patient on corticotropin who developed severe depression His weight fell right down and his blood pressure dropped When he was taken off hypotensive drugs it went up only a little In the autumn of 1958, and I do not think that it was due to his going onto prednisolone, the depression disappeared (he had been treated by a psychiatrist for about a year)

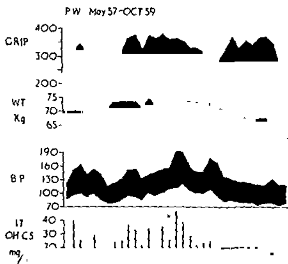


Fig 6

Hypertension developing during 23 months of corticotropin therapy and a fall in blood pressure on transfer to a clinically more effective dose of prednisolone

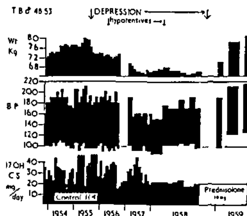


Fig 7.
The effect of therapy and depression upon blood pressure and weight Corticotropin therapy (i e daily I M injections) was given for the first 5 years

and when his depression disappeared his blood pressure shot right up, as did his weight. It is an example of how depression can hold down a blood pressure in spite of steroid therapy, presumably by a central nervous mechanism, and it is also an example of a change from ACTH to prednisolone (small doses) with the blood pressure going up instead of going down

Prof. Stuart-Harris: The whole question of blood pressure in relation to this is about as complicated as the whole question of resistance I wonder if Dr Cope or any of the other physicians have got any points they would like to bring up

Dr. Cope: One point did cross my mind in these blood pressure cases There are, these days, a wide variety of orthodox reasons for blood pressure going up Among them is so-called 'essential hypertension' Now it might well be that some of these are latent hypertensives and that you are just revealing it by giving corticosteroids One ought to know, in any case, whether there is a family history of hypertension It is an essential piece of information

Prof. Stuart-Harris: The other thing though is the effect of changing from ACTH to prednisolone It is the same patient under different conditions showing a fall in blood pressure

Dr. Cope: West did show us a rise on prednisolone We don't know which the majority do I don't know whether Dr West could tell us that? Do the majority fall when they are switched to prednisolone?

Dr. West: I have not switched enough My job, I am afraid, is not to study blood pressure I wish someone else would take this up, it would mean a lot of work Someone ought to study this subject, as to why one patient develops hypertension on a given level of steroid therapy and another does not I thought that it might be an interesting lead for somebody

Dr. Davis: We agree that hypertension is more common with ACTH than it is with comparable doses of oral steroid We also agree that in the majority of cases, when you change them to prednisolone, the blood pressure drops fairly smartly, but not always by any means We have found that if you raise the 17(OH)CS excretion above 30, for any length of time, quite a proportion of patients,

about 50 %, show a rise in blood pressure, though not necessarily to hypertensive levels If you keep it up there, keep it above 40 for a few weeks, most of them become hypertensive and it may take anything up to 3–4 months for the blood pressure to fall even when the 17(OH)CS excretion has come down There seems to be some sort of specific effect of corticotropin in this respect, which does not show with other forms of corticosteroid therapy

Dr. West: Would you say, Professor Symington, that they are likely to be producing more corticosterone with chronic administration? Would you suggest that these people are producing more corticosterone with long term adrenal stimulation of considerable degree?

Prof. Symington: I think that we cannot answer that question

Dr. Singer: Would it not be possible, if you are admitting this patient again to study some of the individual urinary steroids?

Dr. James: There is some evidence that long term treatment with ACTH will alter the pattern of the adrenal steroids Jailer was investigating a patient who had been on ACTH for a very long time He showed that the urinary excretion pattern was different from the normal He was comparing his patient with a patient with Cushing's syndrome and he found that this odd pattern in Cushing's syndrome was reproduced in the patient who had been on ACTH for a long time We studied this on several patients with Cushing's syndrome and there was an apparent difference in steroid pattern, as judged by the metabolites that turned up in the urine We then studied a patient who had been on ACTH for 2 years Unfortunately this turned out to be a normal pattern which is different from Jailer's results I think that it is quite possible that the pattern, after long term stimulation, might be different

Prof. Symington: Did Dorfman not do some incubation studies on an adenoma from a case of Cushing's syndrome in which he showed quite a marked production of corticosterone

Dr. Grant. Yes I remember he did report that He suggested that some of the Cushing's cases, that had a 17(OH) corticosteroid within the normal bracket, may have been Cushing's maintained by a high output of corticosterone, but that, I think, was only a speculation It should be possible to determine tetrahydro-S in urine

Dr. Paulsen: I wonder whether Dr West has some information on the urine quantity changes in these patients, because it is quite probable that all these oxycellulose preparations still have considerable anti-diuretic, vasopressin activity The oxycellulose is adsorbing vasopressin

Dr. West. Do you mean, do they pass *less* water? I have not observed that, the outputs are mostly between 1 & 2 litres and some of them between 2 & 3, year after year

Dr. Cates: Well that does not actually exclude it does it? If they have A D H for a long time they adapt to it It is up to the pharmacologists to tell us how much A D H there is in ACTH

Dr. Shuster: At one time we were treating some patients with pulmonary tuberculosis with long term ACTH They tended to become more rapidly oedematous, we thought, than the patients on corticosteroids Their excretion of a water load, though improved from before therapy, was still below normal We wondered whether in fact they showed signs of increased anti-diuretic activity.

Dr West: I thought that the department of biological standards assured us that in COC purified corticotropin that we use now, there was not enough A D H to have any clinical effect

Dr. Stack-Dunne: Well there is a control on the amount that there can be,

I think that it is 0 5 units per 100 units of corticotropin Would that be right Dr. Jones

Dr. Tindall: Yes, it is usually much less than that.

Dr. Stack-Dunne: In some manufacturer's preparations it is up to that quite commonly. I think that it is 0 5 units per 100 units of corticotropin

Dr. Dixon: Is this then something in the way the oxycellulose is used I mean I would be very interested to know whether I can expect oxycellulose to concentrate ADH.

Dr. Paulsen: It does, quite well

Dr. Dixon: I mean there are very marked differences with M.S H You put in a little oxycellulose and the ACTH goes You have to put in quite a lot more to get a high percentage of the M S H

Dr. Beryl Davies: Is growth hormone supposed to be adsorbed on oxycellulose?

Dr. Dixon: No.

Prof. Stuart-Harris: Does everybody feel exhausted? I think that we ought to ease up don't you The bar is open in the Junior Common Room

DIAGNOSTIC USE OF CORTICOTROPIN

G. M. Wilson
University of Sheffield

Corticotropin affords a method of testing the reserve function of the adrenal cortex. It is a general principle that commonly the earliest manifestations of disease appear as a reduction in the capacity of an organ to respond to a situation that requires increased activity. Corticotropin is thus of particular value in cases where the resting adrenocortical function may fall within the lower limits of the normal range but nevertheless some deficiency is suspected. If corticotropin is to be used as a diagnostic aid in this way two important questions arise.

In the first place we must know how much corticotropin is to be given, by what route, and for how long. Our initial tests were done using an intravenous infusion of ACTH given over four hours, but in those days the material was not highly purified and occasional untoward reactions occured. When long acting preparations became available we turned to the intramuscular route and have used either ACTH gel or corticotropin Zn Initially we gave an injection of 40 units on two successive days. It is now our practice to give 40 units at 12 hourly intervals over a period of three days The main difficulty with regard to duration of stimulation arises in connexion with negative results, as it is always possible to postulate that administration of corticotropin over a more prolonged period might have produced a positive result There is evidence that the maximal effect of 40 units corticotropin Zn continues over about 12 hours and that higher doses do not give a greater response (Gold and Starr, 1959)

The second question concerns what should be measured as an indication of the response of the adrenal cortex. The eosinophil count drops precipitately in a normal subject following the administration of corticotropin This may be a useful test particularly when a quick answer is required or facilities for more elaborate investigations are not available. However, there are certain reservations as fluctuations in eosinophil counts do not necessarily reflect changes in adrenocortical function Most investigators prefer measurements of steroid excretion in the urine col-

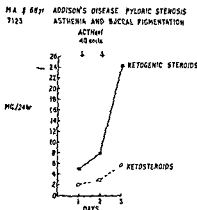


Fig 1.

lected over 12 or 24 hour periods. The 17-ketosteroid excretion is in most cases of little value in assessing the response. We have relied principally on the determination of 17-ketogenic steroids though more recently we have also included measurements of total 17(OH)CS. There are occasional unpredictable fluctuations in the base line excretion and it is important to have estimations over two 24 hour periods before the corticotropin is begun. We have no experience of changes in plasma levels, or of urinary excretion of tetrahydro derivatives or of cortisol following corticotropin administration.

I should now like to turn to certain clinical problems concerning the use of corticotropin.

Diagnosis of Addison's Disease Corticotropin is particularly useful in eliminating doubtful cases. Patients occasionally present with pigmentation, hypotension, asthenia and vomiting secondary to alimentary disorders and the question of adrenocortical insufficiency naturally arises In the presence of cachexia the basal levels of excretion of urinary steroids may be low but there is an adequate response to corticotropin if the adrenal cortex is intact Fig 1 illustrates a case of this type in whom the diagnosis was pyloric stenosis If the excretion of 17-ketogenic steroids rises from these low levels to above 20 mg. in 24 hours, adrenocortical deficiency can be excluded with confidence Patients with Addison's disease never show a rise of this order.

Adrenocortical insufficiency secondary to hypopituitarism. A good response is usually obtained in recent cases, but if the condition is of long duration the adrenal is not readily stimulated (Fig. 2). Further-

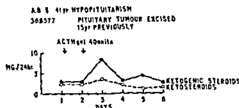


Fig 2.

more hypothyroidism is also present and this depresses the adrenal response. Caution is required in administering corticotropin to patients with hypopituitarism as a large sudden rise in the level of circulating steroids, as the result of either stimulation of the adrenal or exogenous administration of cortisone, may precipitate an acute mental disturbance.

Diagnosis of adrenocortical insufficiency after institution of replacement therapy. If a patient is admitted acutely ill, with features suggestive of Addison's disease, it may be necessary to start treatment before an ACTH test can be carried out. However, if the replacement therapy is kept constant the test can be carried out satisfactorily Cases of definite Addison's disease show no response (Fig. 3) However when a negative result is obtained in these circumstances there is the possibility that the replacement therapy may have rendered the glands unresponsive. This problem has been studied in patients with intact adrenal glands receiving large doses of steroids for the treatment of disease.

Corticotropin tests after large doses of steroids The results of corticotropin tests in a patient before and after administration of prednisolone is shown in Fig 4 After 30 days of treatment the response was delayed in onset but not abolished. Other observations have been made

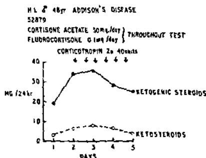


Fig 3.

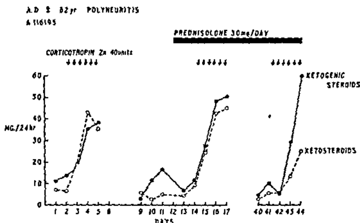


Fig 4

in twelve patients treated for periods of a year or more with high doses of steroids. In the majority of cases an adequate response has been noted but this has often been delayed until the third day. However the results have varied between wide extremes. In one patient who had suffered an adrenal crisis during an attack of gastro-enteritis the response after three days' stimulation was negligible (Fig 5). On the other hand in a patient who had been treated for three years with cortisone, 75 mg. a day, a large effect was obtained. The reason for this variability is not at present clear. In the cases studied it is not consistently associated with the nature or duration of the treatment. There is the possibility that those showing a full and brisk response were negligent in taking the tablets. This is difficult to exclude but seems an unlikely explanation

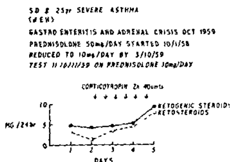


Fig. 5.

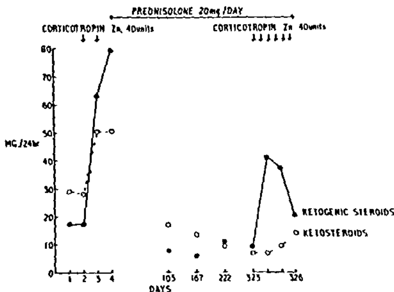


Fig 6.

in those who obtained dramatic relief from their symptoms. The problems associated with this variability in response is being further studied.

Syndrome associated with adrenal overactivity. In Cushing's disease a large response is characteristically seen In the adrenogenital syndrome the ketosteroid excretion is high and is further enhanced by the administration of corticotropin. This response can be partially suppressed by the administration of prednisolone (Fig. 6). In general corticotropin tests are not of great value in the investigation of these cases and the diagnosis is usually established by other investigations

REFERENCE

Gold, E M and P Starr Urinary excretion of 17 KGS in normal subjects before and after stimulation with intramuscular Zinc ACTH J Clin Endocrinol. 19.907-920, 1959

DISCUSSION FOLLOWING PROFESSOR GRAHAM WILSON'S PAPER

Dr. West: Thank you. I am sure there will be no lack of discussion I know that there are a lot of people here who will have many points to raise on these subjects If there is any shortage of discussion I can prompt them, but I think that I will just sit down and wait for people to begin—to carry on with these questions and raise any others that are relevant to the diagnosis of adrenal disorders, and other conditions, using corticotropin

Dr. Chalmers: I just wanted to ask Professor Wilson if he made any further observations on the girl with asthma whose adrenals became unresponsive, or *were* unresponsive. Did you carry on with ACTH over a period?

Prof. Graham Wilson: No We are going to get her in again. She is working now To bring her into hospital for the test requires 5 days, if you give 3 days of ACTH and 2 blank days beforehand If they are working it is a little bit difficult to persuade them to come in for this period This is one of the difficulties You could perhaps arrange it on an out-patient basis

Dr. Cope: On the question of what parameter you should measure when you are giving the ACTH; I can't help feeling that the fact that the eosinophils have gone out of fashion is a pity because they worked a few years ago and there is no reason to believe that they don't still work They have the great advantage of being easy to do and giving you an answer within a couple of hours, or less If you keep your urine specimens in reserve they may save you a lot of work If you get an ambiguous result from the eosinophils you have not lost anything usually, and you can still get some analyses done on the urine Now as regards estimating 17KS in the urine I would entirely agree, I would go further than Professor Wilson, I would say that they are quite useless—for this purpose They may even be misleading and often are But the 17-ketogenic steroids are an orthodox method of estimation that can be done in all hospitals, probably, these days If you get an unequivocal rise in the 17KGS then I think that you have got the answer you want, as Professor Wilson has just said I don't think that one needs to go any further than that except in the rare ambiguous case There still are rare ambiguous cases We have seen one or two, and Thorn has collected, I think, 7 who do not respond to ACTH and yet are not 100 % Addison's disease In other words they have got fixed outputs Now we have got one of these, a man who was a very interesting character He was diagnosed as having Addison's disease and 18 months after that he joined the Army and was in the Guards and went through Dunkirk He was in the army for a long time, about a year, in the Guards—A_1—until he met the M O who diagnosed his original Addison's disease! Whereupon he was promptly boarded-out He followed that up by becoming a water polo player in a crack London team He is [illegible]

On the question of suppression we have looked into a few cases on rather similar lines to Professor Wilson I have not found one yet, who has been on long term steroids, and who has failed to respond to ACTH entirely A number are certainly very sluggish in responding The important question he has raised is of how to investigate the adrenal function of people who are on maintenance steroid therapy. An important clinical question We have done, as Professor Wilson has, ACTH tests whilst people are still on prednisone and we have got the same sort of variation in response that he has Some sluggish rises in activity, some very prompt That may be after 6–9 months or even a year of maintained steroid

therapy. If you have these people on prednisone, as some of Professor Wilson's were, and if you do a quite simple, what I call 'kitchen table' type of chromatography, it is a perfectly easy to check that they are taking their tablets. A lot of the prednisone comes out in the urine free and you can estimate quite easily, you don't need any elaborate chemistry to check that the patients are actually taking their prednisone. I suggest that it is a very easy way of checking With the other analogues the more potent ones, it is much harder because they are present in smaller quantities

Dr. Cates: Dr Cope are you still using the 9-fluoro-hydrocortisone *alone* as your support in studying doubtful "Addisonians"? I saw that you gave prednisone as well I thought that you were the first to advocate maintaining them on 9-alpha-fluoro-hydrocortisone and doing an ACTH test

Dr. Cope: I don't think that it matters what you maintain them on It depends upon what assay method you are going to use If you are going to use 17KGS the prednisone that comes out in the urine and some metabolites will count as 17KGS. (The urinary 17KGS derived from prednisone amounts to approx 50 % of the dose given. Editor) If you use cortisone you are in trouble straight away because you can't distinguish between what has gone in by mouth and what the patient has produced 9-alpha-fluoro-hydrocortisone has the advantage of being in a much smaller dose and therefore interfering much less in any estimation That is the only advantage I think

Dr. Cates: I was trying to find out what is your current practise

Dr. Cope: I have not got one It depends upon the individual patient

Dr. Fotherby: I think that we have got to be clear what we mean by adrenal function If I could show this slide (Fig 1) The adrenal is a fairly complex organ, as Professor Symington showed yesterday By adrenal function we usually mean the production of cortisol and the excretion of ketogenic steroids or 17(OH)CS. I think we should not forget entirely the other steroids that are produced

Response to ACTH

	A Asthma	B Hirsutism	C Hirsutism Hyper-tension	D Ulcerative Colitis	E Ulcerative Colitis	F Ca. Cervix	G Psychoneurosis	H Hirsutism	I Ulcerative Colitis	J Congenital Adrenal Hyperplasia	K Hypertension
17-Oxosteroids	3	2 5	2	2	4	4	3	2	2	1.5	4
17-Hydroxy cortico-steroids	11	2 5	5	5	4	8	9	5	7	2	4
DHA	0–0 34	5	3 5	–	2	–	4	5	10	–	–
Pregnanetriol	10	4	4	–	8	–	5	5	4	0 5	–

Fig I.

Sixty units of corticotropin was given twice daily for two days The figures show the increases in urinary excretion over a predetermined base-line, e g A, 17-oxosteroids: threefold increase over baseline.

by the adrenal The index of function depends upon which steroid you measure This is an odd series of patients whose response to ACTH we have studied The ACTH given was 60 units twice daily for 2 days We have been measuring the 17-oxosteroids—(17-keto steroids), the total 17(OH)CS, dehydroepiandrosterone and pregnanetriol The figures show the increases in excretion over the base-line level, which was determined over three days. If we take subject A we see a threefold increase over the base-line level of 17-oxosteroids, an elevenfold increase in 17-hydroxycorticosteroids and a tenfold increase in pregnanetriol but only a rise from 0 to 0 34 mg in dehydroepiandrosterone.

Prof. Graham Wilson: Dr Sneddon, at the Infirmary here, has investigated several, measuring their daily output over the months In the majority no abnormality has been found (The excretion of 17KGS by hirsute patients)

Dr. Chalmers: Dr Nabarro at the Middlesex has also collected a series and again the majority of them gave responses within the normal range but there is a small group who seem to give excessive responses, who certainly have not got Cushing's syndrome. We have been re-examining them, trying to do a more extensive breakdown of the steroids

Dr. Fotherby: I think that one of the big troubles in this sort of thing is the extent of the normal range You can get between twofold and twentyfold increases There is such a range that if there is an abnormal response it will have to be greatly abnormal to be outside the normal range

Dr. James: Professor Peart has got several of these patients with hirsutism and the only abnormality one can find, as far I can see, is the ratio of the ketosteroids to the ketogenic steroids. It seems to be different in these patients A large number of them, but certainly not all, given an excessive response with ketosteroid output, but a rather feeble response in the ketogenic I don't know what the reason is for this

Dr. Fotherby: Professor Prunty says that it seems to be the aetiocholanolone androsterone excretion that is increased in relation to the total ketosteroids.

Dr. James: Yes, but this is variable as well, sometimes it is the DHA that increases excessively

Dr. West: I would like to produce a slide to illustrate a point We have not fractionated ketosteroids but in the early days we did a lot of 17-ketogenic assays and of course we had to do the ketosteroids with them I went through all these results the other day and produced a slide which I think is relevant to what you have been saying (vide infra)

Dr. Cadman: Whilst Dr West is finding the slide may I add a word to Dr Cope Presumably the eosinophil estimation is of no value in estimating adrenal function of patients who are already on steroids? You have got to use the steroid assays

Dr. Cope: I don't know about that We have not looked into that The original level of eosinophils is an unimportant observation, it is only if it drops If people are on oral steroid therapy there is a wave up and down I think There is a difficulty there, I quite agree I think that there are going to be fluctuations in the level and you may hit a fluctuation and interpret it as a response to ACTH

Prof. Graham Wilson. I suspect that you can get quite a good drop in the eosinophils even though the person is on cortisone if you *do* stimulate the glands

Dr. Cope: But you will have difficulty in interpreting the result

Dr. James: Dr. Cope do you *really* think the eosinophils are an adequate measure of adrenal function? I can think of several normals who have shown no response to the eosinophil depression test and two patients with Addison's disease who did.

Dr. Cope: Not as a final arbiter No

Dr. James: I was going to say that I thought that the sodium potassium ratio in the urine was a better index, a rather more direct one.

Dr. Cope: You are measuring a different thing though. You are measuring aldosterone or something related to it probably, aren't you?

Dr. James: But if you want a response to ACTH, I thought that the aldosterone did not respond and that you would be measuring cortisol response This is really what you want to know— it is sometimes one of the important things—the response in the kidney.

Dr. Cope: To what.

Dr. James: To cortisol This is the difficulty that patients get into, isn't it?

Dr. Cope: You mean the sodium retaining effect?

Dr. James: I would have thought this was a better index of cortisol activity than the non-specific effect on the eosinophils

Dr. Cope: But aren't you getting two things mixed up. One is the response of the kidney to sodium and the other is the cortisol effect

Dr. James: I am suggesting that the sodium potassium ratio in the urine is probably a better index than the eosinophils of cortisol activity—as a measure of response to ACTH

Dr. Cope: I should have doubted that I don't know this one

Dr. Singer: What is the effect of cortisol on the urinary sodium potassium ratio?

Dr. James: You get sodium retention and the sodium potassium ratio in the urine falls very rapidly and this is a better index than the eosinophils as judged by the steroid results after ACTH

Dr. Singer: There is no guarantee of course that aldosterone does *not* increase in short-term ACTH treatment If you give cortisol alone do you get this same effect?

Dr. James: Oh yes, certainly

Dr. Fotherby: The levels of eosinophiles are sometimes so low anyway that it is difficult to tell whether there is a significant fall.

Dr. Cope: Yes, if they are down to 70 per cubic mm that is all right but if they are down to 25 that is a bit difficult Below that admittedly you would be in trouble My point is not that you should use it as the only test but that it is a very quick one If you get an ambiguous result out of it you have not lost anything It only takes ten minutes to do a count.

Dr. James: This is the danger—that the result may not be ambiguous—it may be wrong You may get a patient with Addison's disease who gets a fall in eosinophils after ACTH This could be very dangerous—if you treat them on that basis

Dr. Cope: I know all these things can happen but we have not actually met them We have met the other way round People whose eosinophils failed to drop and who did not have Addison's disease, but then you are treading on the right side. In fact in the early days I was very interested in this because the original tests came out from Harvard where they were using adrenalin I promptly tried it on myself and I was faced with the decision that either the test was completely phoney or that I had Addison's disease That was a very early observation We never had any seriously misleading, clinically misleading, trouble with the eosinophils

Dr. Cates: Of course in clinical practise there is usually no difficulty in making a diagnosis of a pretty straight forward Addison's disease. The difficulty is in weeding out the ones that aren't, but look as if they are The most difficult ones that we have had, and I am sure you have had, have had some small bowel abnormality and have a sort of functional inactivity of the adrenal I find them extraordinarily difficult They have impaired water excretion and other signs—in fact they improve when you give them cortisone

Dr. Cope: The water test yes, quite right, you can get a patient who will improve on a water test in that way But these people, I think that Dr. Shuster may have more information on this, of whom I have come across a relatively small number as 'query Addison's,' often have pigmentation of various kinds and low serum chlorides and low ketosteroids We always find free cortisol in the urine, and that rules out Addison's disease to me straight away They usually excrete quite generous amounts of cortisol.

Dr. Shuster: Yes I think one can distinguish in a single urine without ACTH, though the best test is with ACTH You can test on a single urine for the ratio of unconjugated to conjugated steroid will be high, while in Addison's, if there is any steroid, this ratio will be normal

Dr. Cates: It is quite a specialized investigation that you could not obtain in a peripheral hospital—the conjugated and unconjugated steroid assays.

Dr. Cope: Yes, but they could do the free F. It is a simple technique I have not actually done it on the kitchen table but I think I could The great advantage of this is that you can then wave the spot in front of the clinician and say I garantee that this patient is producing hydrocortisone and there is no argument It is as valid as that You can show him the hydrocortisone Now I find that a tremendous help diagnostically in that sort of case

Dr. West: I am glad that Dr. Cope finds it so easy and hopes to do it on the kitchen table, it has taken us a year to get the method going reliably. Does Dr Paulsen want to say something?

Dr. Paulsen: I only wanted to support the opinion that the eosinopenia is a reliable test We have used it for many years, not for diagnostic purposes, but for our routine evaluation of ACTH preparations and we have found it useful even for quantitative evaluations I think that it is not a very simple method, it has to be done with extreme care of each little detail It has taken us almost 2 years to come to a method We are not using the 50 % decrease, we are using a standard that is giving a complete disappearance of the eosinophils, which is usually done with 40 units of a delayed preparation A normal patient whose eosinophil level varies between 100 & 400, will always give a complete disappearance after 40 I U of this standard preparation.

Dr. Shuster: It is also true when the eosinophils are abnormally raised, for example in patients with active tuberculosis The question of Addison's disease arises and they are having I N A H and have a high eosinophil count The eosinophils fall just as readily as in an normal person, even though the initial count may be 1 to 2 thousand

Dr Paulsen: As to the method for measuring the eosinophils I wonder if a book by Finn Rud of about 500 pages, all on the technique of counting eosinophils, is known in this country It is a very good source of information.

Dr. Cope: I thought it was fairly easy I have not done it for a long time but I did hundreds some years ago, that was with Randolph's phloxine propylene glycol method.

Dr. Ross: Thorn originated the eosinophil test and he gave up counting eosinophils in 1955 He does believe that if the G P in the backwoods can't get urine to hospital then counting the eosinophils is useful in the diagnosis of Addison's disease.

Dr. Cope: These two things are telling you quite different things If you know that the eosinophil response is due to changing levels of hydrocortisone then it is probably the most sensitive test there is and with that proviso I think it is admirable. But it tells a very different story because it tells you a moment to moment level Whereas the urine collection over 12–24–48 hours tells of what

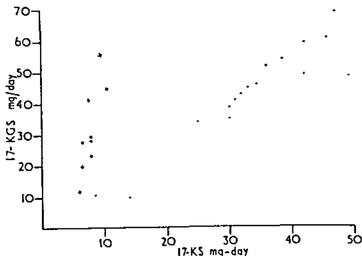


Fig 2

When corticotropin is given the relative increase in 17KS and 17KGS varies from patient to patient This figure illustrates two extremes Both were men and they were of the same age

has happened in the past I think that you have to be a bit careful about what you are reading into the eosinophil The only point I suggest is that a drop to near zero is strongly suggestive evidence, though not proof, that you have got a rise in hydrocortisone, but no more than that. At none of the intermediate stages would I draw any conclusions

Dr. West: We gave it up in 1953 because we were interested in knowing the level of adrenal stimulation within a narrow range, between an output of 20–30 mg of 17(OH)CS We plotted 400–500 eosinophils counts against the output of 17KGS (in these days) and we found no correlation at all We agree entirely that you can pick up a really major stimulation by the eosinophil dropping method I will now show this slide (Fig 2) I would like you to take some thoughts away with you about the ratio of the 17KS to 17KGS and what it means These are two patients, two men of the same age Their 17KGS excretion is plotted against their 17KS excretion These represent two extremes, we find most people lying between You see that in one of these men—I don't know of any difference between them, they were both severe rheumatoids—there was very little rise of 17KS even when the rise of 17KGS was to nearly 60 mg

Voice: Is this on ACTH?

Dr West: Yes on ACTH This is the other chap—his 17-oxosteroids and his 17KGS are almost the same It is so striking that I would like you people who fractionate 17KS to carry on with it and find what this does mean. I wonder whether before we end this discussion anyone would like to say something about SU 4885? It is relevant to the subject this morning. I have not used any If anyone here has used any I am sure that their experience will be of interest to the conference.

Dr. Stowers: It is not a complete block. I have used it in Dundee It only seems to block the output of the cortisol for about 2 days or so and then it spontaneously rises

Dr. Cope: The water test yes, quite right, you can get a patient who will improve on a water test in that way. But these people, I think that Dr. Shuster may have more information on this, of whom I have come across a relatively small number as 'query Addison's,' often have pigmentation of various kinds and low serum chlorides and low ketosteroids. We always find free cortisol in the urine, and that rules out Addison's disease to me straight away. They usually excrete quite generous amounts of cortisol

Dr. Shuster: Yes I think one can distinguish in a single urine without ACTH, though the best test is with ACTH. You can test on a single urine for the ratio of unconjugated to conjugated steroid will be high, while in Addison's, if there is any steroid, this ratio will be normal

Dr. Cates: It is quite a specialized investigation that you could not obtain in a peripheral hospital—the conjugated and unconjugated steroid assays.

Dr. Cope: Yes, but they could do the free F It is a simple technique. I have not actually done it on the kitchen table but I think I could. The great advantage of this is that you can then wave the spot in front of the clinician and say I garantee that this patient is producing hydrocortisone and there is no argument It is as valid as that You can show him the hydrocortisone Now I find that a tremendous help diagnostically in that sort of case

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main symptom was drowsiness. It is an adrenal mitochondrial poison as Professor Symington says but there is no evidence that it influenced mitochondrial reactions in themselves, as in the liver, we checked that. The respiration of the mitochondria appears to be quite normal, but *steroid* reactions in adrenal mitochondria seem to be knocked out.

Prof. Symington: As far as you can see the 17 & 21 hydroxylating enzymes were not affected?

Dr. Grant: They were all right but they are not found in the mitochondria.

Dr. West: Thank you very much. It may be that we will be able to return to this subject in discussion later I think that as we are having coffee at a quarter to 11, we had better stop now. I will hand the chair back to Professor Graham Wilson.

Prof. Graham Wilson: Without more ado I will call upon Dr Houghton.

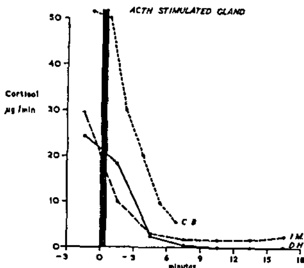


Fig 3

Dr. West: I think that is an extremely important point It is a thing that worried me That if you are testing a person who has an inadequate output or a border line output and you give SU4885 you push them into an Addisonian state What you say is very reassuring

Dr. Stowers: We studied, I think, a dozen or so

Dr. Grant: We have had some experience of this SU4885 and unfortunately, as people have said, Ciba are no longer producing it The British medical representative said that they were after something much better Everybody knows that SU4885 specifically inhibits 11-beta-hydroxylation We have shown that it inhibits other hydroxylations which lead to products that we are not very interested in We just don't know the physiological role of hydroxy compounds like 6-hydroxy- and 18-hydroxy compounds I think that Mr Forrest has a slide that is relevant He cannulated some adrenal veins of patients that were given this drug

Prof. Symington: There does seem to be a definite action on the mitochondria We have used it in the hamster There is a tremendous swelling of the mitochondria

Dr. Cates: It is a poison?

Mr. Forrest: This slide (Fig 3) shows the output of cortisol in the left adrenal venous effluent of three patients who had received prior ACTH stimulation of the adrenal As you see the output of cortisol falls very rapidly after an effective dose of SU4885 The dose here is 15 mg per kilo Dr Grant did 11-beta-hydroxyl ation estimations on these adrenals and they were suppressed.

Dr. Grant: I think that we should make it quite clear that as the cortisol output falls it is replaced by compound S, which is the corresponding compound without the 11-beta-hydroxyl group. There is the danger, I suppose, if you keep this up, of producing symptoms of severe hypertension As for the remark about poison, it is *relatively non-toxic as compared with amphenone* The Americans have kept terminal cancer patients on about 10 grammes a day for days and the

CORTICOSTEROIDS AND CORTICOTROPIN IN THE TREATMENT OF PULMONARY TUBERCULOSIS

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Harefield Hospital, Middlesex

As a clinician I am one of the people at the receiving end. I try to make the best use of the materials which are made available and I observe the results, very largely from the end of the bed

My clinical problem in the treatment of acute pulmonary tuberculosis is quite a different one from Dr. West's problem in rheumatoid arthritis. Whereas he seeks to give the smallest effective dose of corticotropin or steroids for a long period, I seek to give the maximum tolerated dose for a short period If our findings and conclusions differ in some respects this may be one reason

In the treatment of acute pulmonary tuberculosis I try to do two things with steroids to ameliorate allergic reactions and to modify tissue reactions arising from the invasion of the host by the tubercle bacillus.

I would like to use the clinical picture presented by pulmonary tuberculosis to illustrate some of the different potentialities of corticotropin and substitution therapy in achieving these two ends.

I would also mention one or two other uses of steroids during the course of treatment. I think tuberculosis is a satisfactory condition to study, because the patient presents both clinically and radiologically a situation which is rapidly reversible when treatment is effective, and success can be assessed factually.

The histological appearances and behaviour of tuberculous lesions are very familiar. In theory it should be possible to alter this fundamental tissue reaction to the tubercle bacillus by the use of corticosteroids, and indeed, Hart showed in 1950 that the tuberculous process in guinea pigs was enhanced when they were given cortisone There is also considerable evidence that latent tuberculous lesions can be reactivated when cortisone is used in the absence of anti-bacterial drugs. The cellular pattern of the tubercle, which epitomises the tuberculous tissue reaction, is essentially defensive, consisting as it does of rings of epithelioid cells, lymphocytes and fibroblasts, with a surrounding area of inflammation or allergy of varying intensity. The epithelioid cells are

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believed to be associated with granulation tissue formation, and their accumulation may well be suppressed by the action of cortisone. Similarly, lymphocytic, fibrocytic and associated allergic reactions should also be modified. Freed from these natural barriers, the bacilli become more invasive, unless controlled by an effective antibiotic.

When tubercle formation is extensive it leads to tissue damage whether the process proceeds in the direction of caseation or of fibrosis. It is in fact an exaggerated protective reaction which destroys the host tissues, and it is an exaggerated allergic response which accounts for all the classical symptoms of phthisis. The rationale of using steroids in pulmonary tuberculosis depends upon the suppression of these responses, thus minimising damage and at the same time providing a better exposure of the invading organisms to the anti-bacterial drugs. The theoretical advantages of using cortisone in combination with anti-tuberculous drugs are in fact reasonably clear. In practise it is found that recovery is often accelerated in this way and in my view residual lung damage is less. Relatively high dosage of corticotropin af steroid is required.

It could be suggested that acquired immunity would be impeded under cortisone and that natural healing might not occur A colleague and I BCG vaccinated 50 guinea pigs. Twenty five of them were kept under cortisone at the time of vaccination and for six weeks afterwards. The cortisone animals developed papules at the site of vaccination, but subsequent tuberculin response was less than in the control group. All animals then received a challenging dose of live tubercle bacilli, strain R.37 The animals receiving BCG plus cortisone had better protection than those receiving BCG alone. Acquired immunity was therefore not suppressed by cortisone, and at the same time we demonstrated that such immunity was not dependent upon the development of allergy. We subsequently showed that the BCG organisms survived longer in the tissues when cortisone was used, and this may have accounted for the increased measure of immunity which occured

In cases of active pulmonary tuberculosis, which is partly a stress disease, there has always been a suggestion of hypofunction of the suprarenal cortex It has been suggested at this meeting that the idea of adrenal exhaustion is an obsolete conception, and this may be so. But there is some clinical evidence to this effect, and that is one reason why I have preferred not to embark upon substitution therapy in pulmonary tuberculosis. Moreover, in cases in which I have used cortisone or prednisone I have noticed a rebound, that is, a recrudescence of

radiological and clinical manifestations, on withdrawal, and this has been a common experience with other workers. It does not occur when ACTH is used—or not nearly to the same extent.

In this disease there is nearly always a brisk response to corticotropin, and this can be observed in almost moribund patients This response to cortical stimulation is much more apparent than I have found in, for example, cases of asthma, though I cannot support this as yet with figures relating to corticosteroid excretion in the two groups of patients. Why is the stressed adrenal in tuberculosis apparently more reactive than the stressed adrenal in asthma?

I do not intend to discuss clinical aspects of the cases I have treated, but I will mention that I have followed up 100 cases of advanced pulmonary tuberculosis in which ACTH was given for three months together with chemotherapy. The follow up was from five to eight years, and 98 % of patients had no relapse. Evidently the steroid effect on tissue reaction did not prevent ultimate healing Peptic ulceration does not occur in cases of pulmonary tuberculosis treated for three months either with corticotropin or prednisone, with a maintenance dose equivalent to 80 mgs cortisone I believe that most reports of trouble have been in rheumatoid patients, and it seems possible that conditions promoting peptic ulceration may be peculiar to that disease. In tuberculosis indeed, gastro-intestinal irritation due to the drug para-amino-salicylic acid, which is common, can be prevented by a small daily dose of prednisone—5 mgm. is usually sufficient. This would seem to be paradoxical

I have not observed the development of resistance to ACTH although I have used crude material, but my period of treatment is relatively short On the other hand, I have been impressed by the apparent build up effect which has been referred to by other speakers. I have said that I prefer ACTH to steroid administration in tuberculosis. When I first began to use ACTH in 1950 I used a crude preparation which was given four times a day It looked like porridge. It was extremely effective The earlier long acting zinc preparation, Organon Z was satisfactory, but I thought not quite so clinically effective The more recent purified Organon ZN is, in my experience, a relatively impotent preparation as judged clinically and by steroid excretion standards I have always used Organon preparations, and I would like Dr Tindall to comment on this opinion.

I would mention that an extensive trial is being undertaken in this country involving a large number of treatment centres At first the

purified preparation ZN was used, but it became obvious to experienced observers that this was ineffective clinically, and this was confirmed by urinary steroid output estimations. It was decided to revert to the crude Z material for the remainder of the trial. Although I am not in a position to give the results of the trial, I think the difference in potency of the two preparations will be apparent.

I use steroids as part of a rapid desensitisation technique in patients who are hypersensitive to a known allergen, such as streptomycin or PAS. The allergen is given in maximal doses, and the expected hypersensitive response is prevented by a high dose of steroid. Desensitisation is obtained in a few days. It is essential that the steroid cover should be effective. I used to use ACTH, but I found with recent purified preparations that there was often no reassuring drop in total eosinophils, and we failed to prevent the allergic response to the injection of antigen. As some of the patients were asthmatics being desensitised to pollens, this could prove disastrous I now use prednisone or dexamethasone, the anti-allergic effects of which are predictable. This technique is really a very crucial clinical test of the antiallergic properties of ACTH or steroid, but I do not think I should enlarge on this now.

I wish to refer to differences which I have found in certain effects of cortisone and hydrocortisone on the one hand, and prednisone and prednisolone on the other, in experimental animals, in relation to the suppression of tissue reactions. We have found that 5,000 roentgen of X-radiation applied to the skin of a guinea pig over a small area, causes ulceration and necrosis in thirty days The rather complicated early changes are associated with cells proliferation and fibrosis If cortisone is injected daily in very large doses starting with ten days irradiation, no ulceration occurs and the skin remains relatively normal in appearance up to 100 days or longer Subsequently there is some superficial and modified breakdown. Breakdown occurs within days if cortisone is withdrawn prematurely The explanation of this amelioration of radiation damage is obscure, but I think it is associated with the suspension of cell proliferation and fibroblast activity, by cortisone. If prednisone or prednisolone are used in equivalent dosage no such protective effect occurs, the treated animals behaving as the controls Post mortem examination however shows the suprarenal glands to be diminished in size and weight, showing that the prednisone was effectively absorbed In irradiated guinea pig skin therefore, cortisone has protective effects which prednisone does not possess Some indirect confirmation of this dif-

ference in remote effect is given in the French work on the suppression of metastases by the use of cortisone and massive X-irradiation (Ref. 1). Prednisone was not found to have the same effect in this work as cortisone.

We also used S U.4885 as a possible suprarenal cortex suppressor, believing that the reverse phenomenon (enhancement of X-irradiation damage) might be demonstrated, but this was not the case

If a similar failure to modify tissue damage in tuberculous lesions were a possibility, then it would certainly reinforce my preference for the use of corticotropin in this condition. I would have thought too that rheumatologists would have preferred to use an agent which might be expected to reduce connective tissue damage and fibrosis, rather than one which was merely an anti-inflammatory agent. I put forward these suggestions very tentatively indeed in the hope that you will help me with these problems. At the moment it seems to me that this very exact technique of producing precise tissue damage by radiation which can always be held in suspension by a known dose of cortisone, might be a useful method of assay for new preparations But admittedly the conditions of the experiment are highly specialised, and it may be, in any case, that clinicians are chiefly concerned with the anti-allergic properties of steroids.

But in conclusion I would like to ask whether with the reduction of side effects from the cortisone analogues, and with the refinements which have been introduced in the preparation of corticotropin, we may have sacrificed some of their clinical effectiveness and scope.

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DISCUSSION FOLLOWING DR HOUGHTON'S PAPER

Mr. Forrest: You said earlier on in the original guinea-pig experiments, in corticosteroid treated animals, the BCG organisms survived longer. Would this not also happen with the tubercle bacillus?

Dr. Houghton: Theoretically yes, it would and it does, but if you are using an effective anti-biotic you get rid of them In fact your exposure is better under these conditions because you don't have the surrounding barrier of lymphocytes, fibrosis and so on. Therefore your antibiotic has better access

Dr. Stowers: Could you repeat what you said about the French comparison in carcinoma cases of prednisone and cortisone

Dr. Houghton: The general gist of it was that they had irradiated metastases from breast carcinoma using cortisone at the same time and they thought that their results were more satisfactory than if they irradiated them without cortisone. But they could not repear this finding if, instead of cortisone, they used prednisone

Dr. Stowers: What good action did they think the cortisone had?

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Dr. Hemingway: May I ask for the reference?

Mr. Forrest: What dose of cortisone did they give?

Dr. Houghton: I shall have to look this up.

Dr. Cates: Did we hear yesterday, that cortisone inhibited the spread of metastases in the liver?

Dr. Hemingway: Not so much the spread of metastases Cells that have been counted and injected into the portal vein in rats, these cells normally do not develop, they do not become masses of tumour unless the liver is handled thus causing, it is thought, very slight damage to the liver cells. This damage is presumed to put out some substances, yet unknown, which is enabling the tumour cells to develop If they were injected in larger numbers then they would not need this damage to enable them to start off There is a minimal number of cells a colony to develop

Dr. Cates: Where does cortisone come into that?

Dr. Hemingway: It has been found that this can be suppressed by increasing the dose of cortisone. It is believed that the action of cortisone is interfering with these as yet unknown substances that are put out by damaged cells

Dr. Cates: Where is this all from?

Dr. Hemingway: This particular thing is by Fischer & Fischer—I am not quite certain which issue, of Cancer I think that it was three months ago probably

Mr. Forrest: Which tumour was it that they were dealing with?

Dr. Hemingway: Walker 256 carcinoma

Mr. Forrest: In these breast cases it is rather ambiguous at the moment because it is known that prednisone by itself may cause a regression in a hormone dependent tumour *(which they did not find) So this may be a factor that comes* into this apparent increased sensitivity to radioactivity

Dr. Houghton: Should I just mention one thing about this as it seems to be of interest We had a mouse tumour which we were able to implant in the skin of mice, which grew rapidly over a period of about 16 days At the end of that time the mouse had to be destroyed because the tumour was very large We implanted a very large number af these mouse tumours and measured the size of the tumours with callipers—when the tumour was established We then submitted one group of mice to irradiation, I can't give you the dose off hand,

it was a retarding dose, to another group we gave cortisone alone and to a third group we gave cortisone plus irradiation, believing that the cortisone might protect the tumour in the way we had found that it protects the normal tissues from irradiation We had a control group incidentally which grew very rapidly and had to be destroyed in about 12 days. The tumour in the irradiated group grew rather more slowly—I could draw this rather more easily for you—this is very rough indeed—days, size (that is the end of your pencil Grant) if we take—if this is size here and this is days up to about 15 or 16 days the ordinary growth is something like that We found that if you irradiate them you get a slower tumour growth and if you give cortisone you also retard the growth of these tumours But if you give cortisone plus irradiation you get no growth at all—until the final release and then they start to grow again This is the reverse of what we expected to find It did seem that the cortisone enhanced the value of X-irradiation in these tumours

Prof. Graham Wilson: Thank you very much I am very sorry to cut this discussion short but I think that the choice is between going on discussing and cold coffee We would like to resume at 11 o'clock

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THE COMPARISON OF LONG TERM CORTICOTROPHIN AND ORAL CORTICOSTEROID THERAPY IN THE TREATMENT OF RHEUMATOID ARTHRITIS

P.S. Davis
West London Hospital Medical School

These observations are based on experience of long-term corticotrophin therapy in Rheumatoid Arthritis at the West London Hospital, which has already been published in part (Annals Rheum. Dis. 1959. 18 100) Up to the time of this report a total of 78 patients had been treated since 1951. Regular estimations of urinal total 17-hydroxy corticosteroid (17(OH)CS) excretion have been performed since 1955. The observations on clinical response in the report were confined to a group of patients in whom these estimations and regular clinical assessment were carried out from the commencement of treatment, and who had been followed up for at least two years

The selection of patients and method of using corticotrophin may have influenced our results significantly and are therefore briefly described —

Selection. Our indication for corticosteroid therapy in any form is severe active Rheumatoid Arthritis which has failed to respond to rest, salicylates, and in most cases gold These patients have all been disabled to an extent that they would be unable to continue their occupations unless the disease could be suppressed The only factors that have influenced the selection of patients for corticotrophin rather than an oral corticosteroid have been ability to continue self-injections after leaving hospital and being able to attend the out-patient clinic at monthly intervals.

Method All patients have been admitted to hospital to initiate treatment and have had at least one week's rest whilst base-line assessments were made If there has been no significant improvement with rest, daily injections of corticotrophin gel have been started (given in the evening) and daily estimations of urinary 17(OH)CS excretion carried out Whilst in hospital patients have been taught how to collect and measure a 24-hour urine specimen and the technique of self-injection. After leaving hospital they have sent an aliquot of 24-hours urine to the laboratory each week and have attended the out-patient clinic about once a month for clinical assessment.

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The selection of patients and method of using corticotrophin may have influenced our results significantly and are therefore briefly described:—

Selection. Our indication for corticosteroid therapy in any form is severe active Rheumatoid Arthritis which has failed to respond to rest, salicylates, and in most cases gold These patients have all been disabled to an extent that they would be unable to continue their occupations unless the disease could be suppressed The only factors that have influenced the selection of patients for corticotrophin rather than an oral corticosteroid have been ability to continue self-injections after leaving hospital and being able to attend the out-patient clinic at monthly intervals

Method. All patients have been admitted to hospital to initiate treatment and have had at least one week's rest whilst base-line assessments were made. If there has been no significant improvement with rest, daily injections of corticotrophin gel have been started (given in the evening) and daily estimations of urinary 17(OH)CS excretion carried out. Whilst in hospital patients have been taught how to collect and measure a 24-hour urine specimen and the technique of self-injection After leaving hospital they have sent an aliquot of 24-hours urine to the laboratory each week and have attended the out-patient clinic about once a month for clinical assessment.

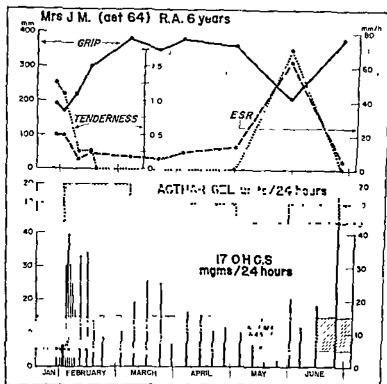


Fig 1

We have not attempted to maintain any given level of adrenal stimulation, but have regulated the dose of corticotrophin primarily by the clinical response whilst watching the 17(OH)CS excretion. hoping in this way to avoid overstimulation and minimise side-effects It appears that on occasions mild adrenal stimulation may be produced without raising the 17(OH)CS excretion above the normal range

Slide 1 (Fig. 1) shows a dramatic clinical relapse after discontinuing corticotrophin, although 17(OH)CS excretion had not apparently indicated any significant adrenal stimulation during the preceeding month. Resumption of treatment produced an equally dramatic improvement

Corticotrophin has been given once daily (in the evening) as a rule Twice daily injections have been used only occasionally for limited periods to intensify the effect

Results of Treatment In the group of 40 patients referred to above, corticotrophin was discontinued before the end of two years in seven because of "acquired resistance" to corticotrophin and in six because of

Results of Test of Strength of Grip.

Trial	No of Cases	Mean Age (yrs)	Mean Duration of Disease (yrs)	Means of Sum of Grip of Both Hands (mm Hg)		
				At Start	After 1 year	After 2 years
Present Study......	27	40 7	4 5	253	457	445
M R C /Nuffield Cortisone (1955)	30	45	0 5	272	389	373
Aspirin	28			227	330	322

Results of Erythrocyte Sedimentation Rate Estimations (mm./hr.)

Trial	No of Cases	Mean Age (yrs)	Mean Duration of Disease (yrs)	Mean E S R (mm /hr)		
				At Start	After 1 year	After 2 years
Present Study (Westergren)	27	40 7	4 5	43	13	18
M R C /Nuffield Cortisone (Wintrobe)	30	45	0 5	42	27	29
Aspirin	28			42	35	28

Fig 2

Treatment of rheumatoid arthritis Comparison of grip strength and erythrocyte sedimentation rate in trials of corticotropin (Present study), cortisone and aspirin.

side-effects (hypertension in two cases, severe acne in two, excessive weight gain in one and mental disorder in one). All these patients were changed on to some form of oral corticosteroid therapy and cannot therefore be included in the results Among the remaining twenty-seven patients, corticotrophin was discontinued in a further six because of the favourable course of the disease. Clinical observations was continued in these cases which are included in the results

Slide 2 (Fig 2) shows the mean figures for E S R and strength of grip, which we have found to be the clinical assessment which most closely reflects disease activity, over a period of two years. In the absence of a true control series, the results obtained in the M.R C /Nuffield Cortisone-Aspirin Trial are shown for comparison. It should be noted that the cases selected for the Cortisone-Aspirin Trial were, on average, earlier ones (average duration of disease 0.5 years as against 4.5 years) and would therefore be expected to have a better natural prognosis. Despite this,

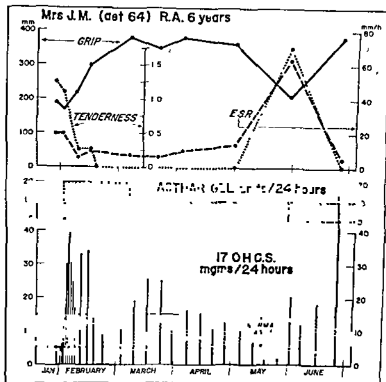


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Changes of X-Ray Appearances of Hands

Treatment Group	No of Cases	After 1 year			After 2 years		
		Deterioration	No Change	Improvement	Deterioration	No Change	Improvement
Corticotrophin	23	3 (13%)	15	5	8 (35%)	10	5
MRC/Nuffield (1959)							
Prednisolone .	41	7 (17%)	30	4	17 (41%)	20	4
Analgesics . ..	35	17 (49%)	17	1	26 (74%)	6	3
MR/CNuffield (1955)							
Cortisone	16				10 (62%)	6	
Aspirin .	16				12 (75%)	4	
MRC/Nuffield (1957)							
Cortisone..	24	10 (42%)	14				
Prednisone .	27	9 (33%)	18				

Fig 4

Treatment of rheumatoid arthritis The changes in the radiographic appearances in the hands of a group of patients treated with corticotropin compared with the changes found in other studies.

(Fig. 4) shows the changes observed in some of the published trials of corticosteroid therapy.

In the recently published results of the M R.C /Nuffield Prednisolone-Analgesic Trial (Ann. Rheum Dis. 1959. 18, 173) it was shown that in the Prednisolone treated group progression of X-ray changes was much less than in the group receiving analgesics. At the end of two years deterioration was evident in the hand X-rays in 41 % of the Prednisolone group as compared with 74 % of the analgesic group. In the M R.C./Nuffield Cortisone-Aspirin Trial comparable X-rays before the start of treatment and after two years were available in only sixteen patients in each group Among these 65 % of the Cortisone group and 75 % of the Aspirin group showed progression of erosion after two years.

We now have a group of twenty-three patients in whom we have been able to compare the hand X-rays before and after two years' continuous corticotrophin therapy. Of these 35 % have shown some increase in erosion after two years, a figure which compares favourably with that of the Prednisolone treated patients.

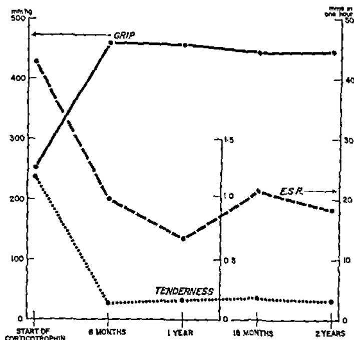


Fig 3.
Data from corticotrophin treated group (see fig 2) set out geographically

the corticotrophin cases show a greater degree of improvement from very similar initial levels, and this improvement is, if anything, better maintained during the second year of treatment.

Slide 3 (Fig. 3) shows this persistance of effect in the corticotrophin treated cases graphically.

It is not possible to make a direct comparison of the dosage used in the cortisone and corticotrophin cases, but some information may be obtained from the average daily excretion of 17(OH)CS In the Cortisone-Aspirin Trial the average daily dose of cortisone acetate was 80 mgms during the first year and 75 mgms. during the second. In our group of patients treated with corticotrophin the average daily excretion of 17(OH)CS was 13.9 mgms during the first year and 17 0 mgms. during the second, which, if one assumes that about 40 % of the adrenal output of cortisol is estimated by this method, would be approximately equivalent to 55 mgms. and 70 mgms. of Cortisone Acetate respectively

X-Ray Changes. X-rays of the hands and feet give the most objective evidence of the progress of the disease in Rheumatoid Arthritis· Slide 4

side-effects. The upper chart shows the titres immediately before changing and the lower after twelve months on an oral corticosteroid. There is a definite increase in titre during this period, although the mean E.S.R. has not changed

Withdrawal. I hope that the evidence I have presented can be accepted as demonstrating that the results of corticotrophin therapy in Rheumatoid Arthritis are as good as, and possibly better than those obtained from oral corticosteroids Quite apart from this, however, we have been impressed that corticotrophin has a great practical advantage over oral corticosteroids in the relative ease of withdrawal

Withdrawal of oral corticosteroids, even if gradual after a relatively short period of treatment, may cause unpleasant, sometimes dangerous, symptoms of adrenal insufficiency. Even if these symptoms do not occur, the process of withdrawal appears not infrequently to cause an exacerbation of the disease. This seems most likely to occur in cases in which the natural activity of the disease has subsided, but there has not been a true remission. Experience of this sort has undoubtedly influenced the indications for corticosteroid therapy in Rheumatoid Arthritis since it must

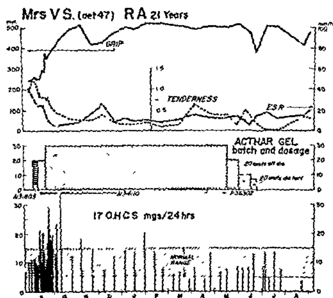
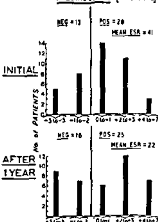
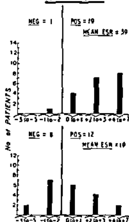


Fig 6

To illustrate a remission of rheumatoid arthritis during corticotrophin therapy.





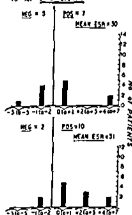


Fig 5.
Treatment of rheumatoid arthritis See text

Sheep Cell Agglutination Tests. (Fig 5).

Despite the favourable effect of Prednisolone on X-ray changes that was found in the Prednisolone-Analgesic Trial, the titre of the sheep cell agglutination test tended to rise during treatment in this group of patients, an observation which some authorities consider may possible indicate a poor long-term prognosis

Unfortunately we have not carried out sheep cell agglutination tests routinely until the past two years and our figures for corticotrophin treated patients are therefore small In a group of twenty patients, however, in whom the D.A T /(SCAT) was done before treatment and after one year, there was a definite fall in titre In the thirteen of these cases where we have been able to repeat the D.A.T. after the second year, there has been no further significant change, during the second year of treatment. This difference in effect between Corticotrophin and Prednisolone does not seem to be due to relatively greater dosage producing more complete suppression of disease activity, since as shown on the chart, the effect on the E.S R. was almost identical in each group.

The third set of figures on this slide shows the changes in sheep cell agglutination titre in twelve patients who were changed from Corticotrophin to oral corticosteroid because of acquired resistance or particular

Incidence of Side-Effects.

Therapy		Oral Steroids 104		Corticotrophin 78	
No. of Cases		No.	Per cent.	No	Per cent.
Side-Effects	Weight Increase	29	27 9	24	30 7
	Moon Facies	54	51 9	42	53 8
	Oedema	34	32.7	23	29.4
	Dyspepsia	49	47 1	12	15.3
	Peptic Ulceration (or Erosion)	12	11 5	2	2 5
	Glycosuria	5	4 8	10	12 8
	Acne	7	6.7	25	32 0
	Hirsuties	20	19.2	17	21.7
	Pigmentation	1	0 96	17	21.7
	Chemosis	3	2 9	6	7 6
	Menstrual Irregularity	11/76	14.3	13/50	26 0
	Hypertension	18	17 3	26	33 3
	Osteoporosis	6	5 8	4	5 1
	Mental Disturbance	2	1.9	3	3 8

Fig 7 See text

also occured not infrequently, usually only to a slight degree, although acne has been bad enough in two cases to necessitate changing from corticotrophin to an oral corticosteroid

THE DIFFERENCES BETWEEN ORAL CORTICOSTEROID AND CORTICOTROPHIN THERAPY

Dr. West: In opening this discussion I wish to point out briefly the obvious differences and mention some others which, I think, are becoming apparent though for which there is not, as yet, adequate objective evidence

Oral corticosteroid therapy differs from corticotrophin therapy in the manner in which it reaches the systemic circulation and in the fact that it reduces or stops the adrenal secretion of certain steroids that are normally secreted with cortisol Theoretically it is not a good thing to pass a steroid hormone in high concentration through the intestinal wall and liver in order to distribute it to the body tissues Also, theoretically, it is unwise to suppress any of the secretions of any gland unless one is quite sure that they have no physiological function

Corticotrophin therapy differs from oral corticosteroid therapy in that it *increases* the secretion of the aforementioned adrenal steroids. Corticotrophin also affects the

be accepted that, once started, corticosteroids may have to be given indefinitely.

In our experience corticotrophin has proved more satisfactory in this respect. As well as the six cases already mentioned, corticotrophin has been withdrawn because of the favourable course of the disease in another nine cases, making a total of fifteen out of seventy-eight cases. In none of these has there been any evidence of a withdrawal syndrome or significant "rebound" of disease activity, despite the fact that in the majority the disease was not in remission but was sufficiently quiescent to be controlled with occasional doses of salicylate. *Slide 6* (Fig 6) illustrates such a case.

Although exogenous corticotrophin therapy causes adrenal hypertrophy rather than atrophy, it would be expected to suppress endogenous corticotrophin production in the same way as exogenous corticosteroids. It seems possible, therefore, that the absence of any evidence of such suppression in our cases may be due to the corticotrophin being administered only once daily. The duration of effect of the gel preparations appears to be only twelve to sixteen hours so that one could postulate that endogenous corticotrophin production is only intermittently suppressed.

Side Effects The value of any particular form of corticosteroid therapy, especially in long-term treatment, depends on the relative suppressive effect in the disease as compared with side-effects In our experience the overall incidence of side-effects has been very similar with corticotrophin and oral corticosteroids, but the relative incidence of particular side-effects has differed significantly. *Slide 7* (Fig 7) illustrates this point.

Firstly, some comment should be made about the apparently high total incidence of side-effects in both groups: This I think must be due to our close observation and strict criteria in assessing the cases In all probability we have included cases with minimal signs that might be overlooked in routine clinical practice In fact the average doses of oral corticosteroids we have used do not differ significantly from other published series and the overall incidence of side-effects should not therefore be very different In any case it is the relative incidence of certain side-effects that is of particular interest. The incidence of gastric side-effects which are of great practical importance with oral corticosteroids, is considerably lower with corticotrophin. On the other hand, hypertension occurs more frequently and in our experience what we have termed "androgenic" side-effects, especially acne and menstrual disturbance, have

treatment, are less subject to the 'easy exhaustion' of corticosteroid treated rheumatoids, are less prone in the post-menopausal years to hot flushes, and are less liable to a decrease in libido All these refer to *long term* therapy

Dr. James: May I just point out some of the biochemical differences between steroid therapy and the ACTH therapy As is well known if you give hydrocortisone or one of its analogues you effectively suppress the pituitary and therefore the adrenal secretions So if you have a urine from someone receiving say hydrocortisone you find just the metabolites of hydrocortisone and to a small extent the other metabolites that are from the adrenal androgen If you give ACTH you stimulate the production of hydrocortisone and corticosterone but also in the urine you find the four other metabolites that are derived from adrenal androgen Now what the function of this adrenal androgen is normally one doesn't know but at high levels is has, by definition, androgenic effects and also an anabolic effect Now the corticosteroids like hydrocortisone have a protein catabolic effect and there is clearly a difference here on protein metabolism This may account for some for the differences that one finds in therapy I was wondering if there was any case for giving patients, who are on steroid therapy, some of these anabolic agents as well—to try to counteract the catabolic effects of hydrocortisone These anabolic steroids are now non-virilizing and the undesirable androgenic effects that you get with corticotrophin therapy might be obviated in this way

Dr. West: May I speak to that? We have been concerned with this problem We had some Nilevar (17-ethyl-19-nortestosterone) given to us and started a controlled trial in wasted rheumatoid patients We ran this for six months with a comparatively high dose, 30 mg a day, and we measured the creatinine excretion as an index of muscle mass—as we thought After six months the creatinine excretion had risen, in one patient it had risen 50% Those patients in the trial who turned out have been on Nivelar, all had markedly raised creatinine excretions yet their muscles did not appear any stronger or bigger Three months after stopping the therapy their creatinine excretions had dropped to where they were before and the patients looked exactly the same as they did before Their muscles did not appear to have diminished and their strength of grip had not gone down So I am rather mystified by anabolic steroid therapy Of course we do not use *big catabolic* doses of prednisolone

Prof. Graham Wilson: Did their weight change?

Dr. West: Yes their mean weight went up by 3 or 4 Kg at 3 months and it dropped down to an increase of about 2 3 Kg at 6 months

Prof. Symington: Dr West did mention this question of the stiffness of the rheumatoid patient in the morning I think that it is generally accepted that we have diurnal variation in the normal adrenal and that it is more active at 6 o'clock in the morning Is there any diurnal variation in the adrenal of the rheumatoid Have you investigated this?

Dr. West: We did years ago and found that it was the same as in normal individuals

Prof. Symington: And yet they are stiffer in the morning?

Dr. Cope: I think that this general impression that West is voicing now, that corticotropin therapy is better than corticosteroid therapy, is widespread in clinicians all over the world It is in the literature in all sorts of contexts I have never actually seen a statement to the reverse, that steroid therapy in any condition is better than corticotropin It is an indefinable one quite often and it may well be that James' suggestion is a highly relevant one That the androgens—

RHEUMATOID ARTHRITIS X-RAY CHANGES

Erosions

Therapy	No	Period	No Advance	Advance
Analgesics .	34	2 yr	26%	74%
Prednisolone . .	41	2 yr	59%	41%
Corticotropin . .	19	3 yr	68%	32%

Major advance

Therapy	No.	Period	%
Analgesics .	9	2 yr	55
Prednisolone .	10	3 yr	20
Corticotropin	19	3 yr	10

Fig I.
Upper box.—data from MRC/Nuffield analgesics v prednisolone trial and authors corticotropin patients Lower box.— authors own trial patients.

metabolism of many tissues directly although, experimentally, it is necessary to give very large doses before such effects can be measured

Another obvious difference is between the effect of cortisone acetate or cortisol and adrenocortical stimulation (with corticotrophin) upon the course of rheumatoid arthritis I hope that some suggestions will be forthcoming as to why this should be, bearing in mind the fact that oral prednisolone is also much superior to oral cortisone acetate therapy (see Fig 1) The last obvious difference concerns the incidence of peptic ulceration It is known that wound healing is delayed by abnormally high levels of corticosteroids, so it is reasonable to assume that healing gastric erosions may be affected by high local concentrations produced in the stomach when corticosteroids are taken orally Another possible reason is that our patients on corticotrophin therapy have their injections in the evening and do not need to put aspirin-containing analgesics into empty stomachs in the morning to enable them to 'get going'—as corticosteroid treated patients usually do! The corticotrophin treated patients usually need aspirin at tea-time when the effect of their daily injection is wearing off

The differences that are still unproven are as follows

(1) *Bruising* In rheumatoid arthritics treated for several years with conservative doses of corticosteroids (Prednisolone 10 mg Triamcinolone 6 mg Dexamethasone 1 25 mg) one of the most unpleasant side-effects is an exaggeration of their usual tendency to bruise *very easily* These bruises may lead, on legs, to ulceration. It is my impression that corticotrophin treated patients are not subject to this *severe* bruising I am hoping shortly to have sufficient data to confirm this point

(2) These other differences are lesser impressions that call for much more study They are that corticotropin treated patients show less adaptation to their

treatment, are less subject to the 'easy exhaustion' of corticosteroid treated rheumatoids, are less prone in the post-menopausal years to hot flushes, and are less liable to a decrease in libido All these refer to *long term* therapy

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some androgens—possibly unknown ones, come into this Now the other thing that I would like to raise, because I think that this is highly relevant and a major problem always, this hasn't been touched on, and is the criteria for stopping steroid therapy in any state. Presumably your rheumatoid arthritis is the major one How do you decide to stop it and under what conditions Are you continually doing tentative withdrawals and then if they flare up do you put it back Do you go on indefinitely like that

Dr. West: Speaking for rheumatoids that is more or less what we do There is a point that I would like to add to this There are some patients when we withdraw—say when we go down to 7½ mg. of prednisolone and then to 5—in whom we don't know whether their symptoms are entirely rheumatoid coming back or whether there is adrenal insufficiency added. What we are doing now is, when we get down to 5 mg, we do a 17(OH)CS output to see how much their adrenals are contributing If it is contributing a nice lot, let us say on 5 mg of prednisolone the output is 10 mg, we know then that their adrenals are contributing almost up to the normal amount But if we find that the output is only 5 mg we are very hesitant in going any lower because we may be going to have the patient back next week or in a week or two with the signs of hypo-adrenalism. (The urinary recovery of administered prednisolone in mg of 17(OH)CS is about 45 %)

Dr. Cates: But what about ACTH?

Dr. West: *Withdrawing?*

Dr. Cates: You were talking about the withdrawal of prednisolone. Dr. Cope asked about the withdrawal of ACTH

Dr. Cope: Yes well I was talking about the withdrawal of either really but ACTH would be a different problem

Dr. West: We work entirely on the sedimentation rate If the sedimentation rate is normal, and the disease apparently inactive, we gradually lower the 17(OH)CS output down and down until it is 12 or 13 mg Then usually we can stop treatment altogether

Dr. Dixon: What would happen if you were to give ACTH for about 4 days just before withdrawing oral steroid Might that help the patient's adrenal?

Voices: In some it does and in others it doesn't

Dr. Dixon: It takes longer than that to build up a long term suppressed adrenal?

Dr. Davis: I think that it is not really the adrenal that is suppressed, very often As Dr West has shown (1953) with patients on long term steroid therapy you can still produce a brisk adrenal response with exogenous ACTH Even while they are still having it I can remember three of our patients who have responded well to a course of ACTH during cortisone withdrawal and despite this they have gone into a chronic mild cortisone withdrawal syndrome The interesting thing is that during that phase, with a low 17(OH)CS excretion, they have appeared to be hypersensitive to ACTH

Dr. Dixon: So you think that it is either the pituitary or the state of the hypothalamus?

Dr. Davis: Yes It isn't necessarily the adrenal that fails to respond

Dr. West: Can anyone help us on this We need a uniform, safe, stressor of, presumably, the hypothalamus, for testing the response of these people I know TAB can be used but I would like to meet somebody who has used it and knows whether or not it can be given intravenously, safety, to *these people*

Dr. Fotherby: We have used TAB in four patients. Using 5 million organisms per ml *intravenously*, one single injection, the response that we have got has been equivalent to that produced by 60 units of ACTH approximately It is a

fairly quick response In the four patients that we have studied so far, it has had no adverse effect, but I have no doubt that complications may occur

Dr. Davis: Did they in fact respond? What happens if they don't respond?

Dr. Tindall: As a matter of fact purified bacterial pyrogen is entirely predictable and can be used to produce adrenocortical responses The dose required to produce a really definite thermogenic response can be predicted A very small amount—and the therapeutic index is enormous

Dr. Cates: That does not get over the difficulty that if you have someone who is not going to make corticoids when you give him a fever you may be doing a dangerous trick.

Dr. Stowers: TAB used to be used in the treatment of rheumatoid arthritis I remember, 18 years ago in the States, administering this therapy. Presumably its action was by producing cortisol

Dr. West: It does Alan Dixon, of the Postgraduate School, got his wife, who was a nurse, to give him some intravenous TAB one week-end five years ago He sent us all the specimens from before and afterwards and from his dose on Saturday and Sunday his output shot up to about 40 mg (17(OH)CS) on each day

Dr. Longson: We have given patients prednisolone therapy for three years and at the end have given ACTH until that produced an increment of about 40 mg of 17KGS; so we felt that they had at least got a responsive adrenal After 2–3 days, their 17KGS having fallen to the 3–4 mg range, we have given SU4885 and have had no response at all We have interpreted that as being a case of pituitary suppression (? permanent) at that stage Jailer has just published some similar cases (four), three of them did respond to SU4885 and one didn't So I really don't know what the answer is What I wondered was, when you give people chronic corticotropin therapy and then you withdraw it, do the urinary 17KGS fall to very low levels, suggesting adrenal suppression, or do they just remain in the normal range. I think that is rather important

Dr. West: The trouble there is that we withdraw it *slowly* We get an output of 17(OH)CS say down to 12 mg we don't know then whether that is the ACTH we are giving or the patients own ACTH

Prof. Graham Wilson: Dr Davis I think that you showed 2 or 3 very low levels, below your normal range

Dr. Davis: Yes, we have some that have gone down below the normal range but most of them settle out in the normal range I was going to say, in answer to Dr Cope, that withdrawing corticotropin in practice is usually quite easy The patients take themselves off in a sense They tell you when they are ready to come off because as you reduce their dose their 17(OH)CS excretion falls gradually and they are no worse You know that when you remove the last little bit of exogenous ACTH they are going to remain the same

Dr. Cope: There is one important point that might clarify here Is it the impression of people who are using much corticotropin that you can withdraw it without rebound hypoadrenalism?

Dr. Davies: Yes *

Dr. Cope: If you are inhibiting ACTH in both groups it is difficult to explain that on physiological grounds

Dr. Davis: That is what I was saying at the end of my talk It is difficult to understand on first principles I wonder whether it is that using the gel preparation once daily, as we have, you are in fact only causing an intermittent suppression of the pituitary, only lasting for about 12 hours.

* *Editors remark* This is not always so in the editor's experience.

Dr. Longson: There is some evidence that steroid therapy and corticotropin therapy are quite different as far as their effects on pituitary ACTH content goes In the last few months there have been 2 or 3 papers on this in which it was shown that if you give steroids the ACTH content of the pituitary is reduced very much, but that if you give ACTH therapy the ACTH content of the gland is increased. That occurs in normal and in adrenalectomized animals The two may not be comparable We had one patient who had received steroid therapy for twelve months, and when we withdrew the steroid he had a very nasty withdrawal reaction The urinary steroids fell to zero We stimulated the adrenal with corticotropin and then withdrew the corticotropin and again the urinary steroids fell to zero We did this three times in this patient. This suggests some permanent pituitary suppression

Dr. Paulsen: Two years ago I went thoroughly through all the published evidence on the question of whether ACTH treatment inhibits pituitary production of ACTH All the papers published up to 1956–7 can be disregarded because they were using methods for ACTH determination that are not sufficiently acceptable At that time I asked Dr Gemzell of Stockholm if he would study the question in animal experiments We did not believe at the starting point that there was a real inhibition of endogenous ACTH production He found in adrenalectomized rats after ACTH-treatment a certain decrease of the ACTH content in the pituitaries, of about 30 % or 40 % But if one considers that in adrenalectomized animals the pituitary content of ACTH is always increased to double it could be calculated that the ACTH content still is higher, after ACTH treatment, than in normal animals. A recent study by Jailer in America, that was done in normal animals, confirms this point of view. Not only was there no decrease, there was a definite increase in ACTH content in the pituitaries of ACTH treated rats

Dr. James: We did some urinary steroid estimations on two patients who had an adrenocortical adenoma and they were very high to start with Then the gland was removed and following operation their steroid levels dropped down to very low values. So we gave ACTH and they responded very well They went up to 20–30 mg of 17(OH)CS but as soon as this was stopped they fell down to very low basal levels again This continued for three months in one case and in the other for very nearly a year so it certainly looks as though their pituitaries had been suppressed for a very long time

Dr. Cope: Do you know whether any adrenal stimulation occurs when you give a dose of gold

Dr. Davis: Not measurably by 17(OH)CS assay We have noted that on a number of occasions

Dr. Paulsen: Has anyone an idea of any association which may connect the fact that gold is found normally in the pituitary gland, with the therapeutic use of gold I think that it is a very peculiar co-incidence that the pituitary gland is the only tissue in the body where gold is found

Dr. Shuster: Is there no purpura in corticotropin treated patients? Is this an absolute thing?

Dr. West: Have we seen no purpura in corticotropin treated patients? (Yes) I was not talking about purpura, I was talking about bruising

Dr. Shuster: Those large bruises on the arms and legs (Yes) We probably mean the same thing

Dr. West: Yes I have seen them in the legs in *one* patient I have been looking for them all this (last) year

Dr. Shuster: This is a very big reduction in incidence (compared with corticosteroid therapy) and in so far as the cause of corticosteroid purpura appears

to be related mainly to atrophy of dermal collagen, in addition to an inhibition of inflammatory response, this perhaps supports the idea that androgen suppression is responsible

Prof. Graham Wilson: Have you got this on a quantitative bases Have you done any capillary resistance tests on corticotropin therapy and steroid therapy?

Dr. West: No we haven't. The person who has been working on these tests is Duthie in Edinburgh He did tests 4–5 years ago on rheumatoids and showed that their capillary resistance was reduced, then he put them on steroids and their resistance went up I have suggested that he repeat all these again on any patients who are still on steroids, to see if it has dropped down again

Prof. Graham Wilson: I think that his work was done on a acute basis Just a few days of treatment and it showed no change Obviously it would be much more interesting to do it on people after a long period of treatment

Dr. Shuster: We have done some on people on long term therapy Cortisone or any corticosteroid therapy raises the critical pressure Despite this they still have this very gross purpura The reason being that the straight suction of pressure within the vessel does not produce it It is a shearing stress to the vessel that produces the purpura There is the atrophy of the dermal collagen, the two layers, as in senile skin, can slip one on the other, and a lateral shear will produce the purpura Then there is the second problem of the resorbtion which is just a matter of the inhibition of the inflammatory response All these patients have a high capillary resistance in the presence of gross purpura

Dr. Stowers: It would be very interesting to narrow this down to one factor operating, because arteriosclerotic hypertensive patients have this capillary fragility If one is going to treat people with steroids perhaps a hypertensive factor comes in as well You have a multiplicity of factors

Dr. Shuster: Yes—I think that you get this in quite young people where there is no question of that

Dr. Stowers. I would like to know whether Dr West feels that the bone erosive changes have been effectively proved The number of cases shown are still relatively small and they were probably selected with other aims in mind We all know how some rheumatoid patients, whatever you do, show progressive erosion of their joints while others don't

Dr. West: In the MRC prednisolone analgesics trial—

Voice: It was blind was it?

Dr. West: It was a controlled trial There were about 76 patients divided into two groups The X-rays were read by three observers and the statisticians correlated their results and were amazed that their findings correlated so well I think that that is what you would want to see done and it has been done

Dr. Paulsen: I wonder whether this question of erosions may be connected with a study made by Jordal, published in the Danish language Perhaps it is not known on this side He made a study of the frequency of osteoporosis in long term treatment with ACTH and corticosteroids and found a considerable difference I think that about 51 % of the cases treated with corticosteroids had signs of osteoporosis, whereas the ACTH treated had only between 15–20 % or so It may be explained in the same way as you have suggested, that there is a difference in the stimulation of anabolic steroids

Prof. Graham Wilson· Well I am very sorry to bring this very interesting discussion to a close I think the time has now come for us to ask Professor Stuart-Harris if he will sum up

Dr. Longson: There is some evidence that steroid therapy and corticotropin therapy are quite different as far as their effects on pituitary ACTH content goes In the last few months there have been 2 or 3 papers on this in which it was shown that if you give steroids the ACTH content of the pituitary is reduced very much, but that if you give ACTH therapy the ACTH content of the gland is increased That occurs in normal and in adrenalectomized animals The two may not be comparable We had one patient who had received steroid therapy for twelve months, and when we withdrew the steroid he had a very nasty withdrawal reaction The urinary steroids fell to zero We stimulated the adrenal with corticotropin and then withdrew the corticotropin and again the urinary steroids fell to zero We did this three times in this patient. This suggests some permanent pituitary suppression

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view, the most *important* problem that emerges Obviously it is one in which a concerted effort to study changes might tell us quite quickly how to *stop* using these drugs, not how to use them. I have been very priviledged to be a witness to all your deliberations and I would like, in conclusion, to say how much I think that we have all been indebted to Dr. West whose energy and enthusiasm has been responsible for drawing us all together.

C. H. Stuart-Harris: I have got to be very brief. When Dr West showed me the programme I thought that perhaps he had allowed a little to much time for discussion but the fact that I think you were prepared to prolong your discussion indefinitely is a very good indication that West was right and I was wrong. It is also perhaps the reason why I am doing the summing up and not one of you because it is quite obvious that not one of you would be allowed to sum up by your collaborators.

I think that you will agree that this method of bringing together people working in different fields is in fact fruitful I think that it creates interest and there is no doubt that the spark of mind striking against mind has been most obvious during this day and a half. We have had yesterday the scientists telling the clinicians their findings and today the clinicians have told the scientists of their findings I never like to classify the clinicians as scientists because it is regarded as being slightly indecent for a physician to be a scientist I hope that this method of doing things has been the right one I thought myself that we started off quite correctly by learning how the development of knowledge concerning chemical structure is being married to that of function A most elegant example of how chemistry can, in fact, explain physiological matters I suppose it really is that all physiology is becoming chemical now Then there are the changes in the gland in which the morbid anatomical approach is being married to that of enzyme chemistry. In both these collections of material I found the problems remaining to be almost larger than those that had been solved This really holds, I think, for each one of us, that we have certain progress to be recorded but there are *more* things remaining that we don't understand I wonder whether in fact this is not really the reason behind this conference If there had not been the problems outstanding there would have been very little point in coming together to discuss them This is the case, I think, with a question as fundamental as Dr West's apparent acquired resistance to corticotropin It seems to me the block in knowledge of what is the action of the compound is that impeding progress in our understanding of why patients stop responding Immunology is one explanation I wonder whether it is an adaptive change in the gland itself, that is tending to resist this prolonged stimulation by an outside agent. Then today in the clinical studies I think that we have been able to show the scientists, through Dr West's studies and those coming from the West London, the enormous advantage in this field of having had, right from the very beginning, an attempt to study what treatment was doing This is so different from almost all the therapeutics—I am sure if Professor Wilson was giving this summing up he would say the same—that there are few instances in medicine in which you can say that a really concerted attempt to study what is being done by the giving of compounds has ever been made—from the beginning Really although you may grumble and say 'oh but 50 patients are needed to do any sort of assay' you have been helped much much more by the clinicians than you might have expected to have been in other fields I find the question of the use of ACTH, *or* steroids *by mouth*, which seems to be so terribly important to a clinician, to be relatively unimportant in this whole body of knowledge To me this is not the very important matter that stands out It is the development of knowledge about glands and hormones that bear on treatment of disease We have to sacrifice logic for logistics in terms of treatment very often This is an example where time will tell quite clearly which is the method which is going to be used A great deal is to be done